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High-Frequency Ultrasound-Responsive Block Copolymer Micelle

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Micelles of a diblock copolymer composed of poly(ethylene oxide) and poly(2-tetrahydropyranyl methacrylate) (PEO-*b*-PTHPMA) in aqueous solution could be disrupted by high-frequency ultrasound (1.1 MHz). It was found that, upon exposure to a high-intensity focused ultrasound (HIFU) beam at room temperature, the pH value of the micellar solution decreased over irradiation time. The infrared spectroscopic analysis of solid block copolymer samples collected from the ultrasound irradiated micellar solution revealed the formation of carboxylic acid dimers and hydroxyl groups. These characterization results suggest that the high-frequency HIFU beam could induce the hydrolysis reaction of THPMA at room temperature resulting in the cleavage of THP groups. The disruption of PEO-*b*-PTHPMA micelles by ultrasound was investigated by using dynamic light scattering, atomic force microscopy, and fluorescence spectroscopy. On the basis of the pH change, it was found that the disruption process was determined by a number of factors such as the ultrasound power, the micellar solution volume and the location of the focal spot of the ultrasound beam. This study shows the potential to develop ultrasound-sensitive block copolymer micelles by having labile chemical bonds in the polymer structure, and to use the high-frequency HIFU to trigger a chemical reaction for the disruption of micelles.

1. Introduction

For drug delivery, the controlled release of therapeutic compounds both spatially and temporally is a major challenge.[1,2] Polymer micelles, liposomes, hydrogels, and microemulsions have been used as delivery vehicles. Among them, stimuli-responsive amphiphilic block copolymer micelles consisting of a hydrophobic inner core and a hydrophilic corona have attracted much attention. This type of nanocontainers can encapsulate various poorly water-soluble pharmaceuticals in the hydrophobic core and be soluble or dispersible due to the hydrophilic corona. Moreover, under external stimuli such as pH change,[3,4] temperature change,[5−11] and light,[12−14] the hydrophobic−hydrophilic balance of the amphiphilic block copolymer can be changed reversibly or irreversibly often due to a change in the polarity of the hydrophobic block, which leads to disruption of the micelles. If some payloads are encapsulated by the micelles, they can be released in response to the stimuli.

Ultrasound is known as a powerful physical modality for spatial and temporal control of drug delivery.[15] Langer et al. proposed the concept of releasing drugs entrapped in a solid polymer matrix under ultrasound irradiation.[16] Recently, ultrasound-triggered release from liposomes,[17] polyelectrolyte microcontainers,[18] multilayered capsules,[19] microemulsions,[20] and micelles[21−27] has been investigated. However, the use of ultrasound as a rational means to control polymer micellar disruption remains largely unexplored. Rapoport first studied ultrasound-triggered drug release using doxorubicin-loaded Pluronic micelles and showed that ultrasound can effectively penetrate deep into the body in a noninvasive way.[21] Generally, ultrasound technology includes low-frequency power ultrasound and high-frequency diagnostic ultrasound. In the case of low-frequency ultrasound that exerts a strong cavitation effect, a long wavelength is difficult to be focused, and when ultrasound passes through the body, the cavitation may destroy healthy and vital tissues. This restricts the practical clinic use of low-frequency ultrasound to some extent. By contrast, with high-frequency ultrasound, the ultrasonic wave can be focused. This means that only in the focal spot the intensity is quite high, while in the other area the intensity can be

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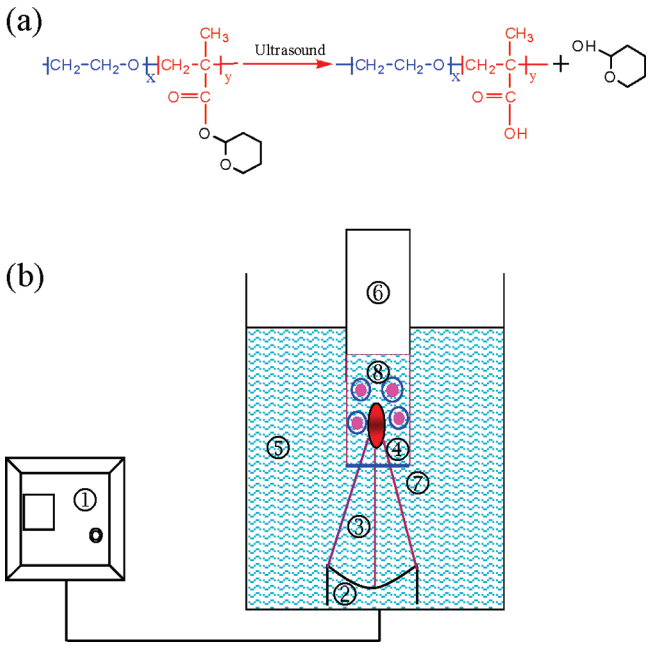

Figure 1. (a) Hydrolysis reaction that can lead to disruption of block copolymer micelles under ultrasound irradiation. (b) Schematic diagram of the used high-intensity focused ultrasound apparatus: ① ultrasound generator, ② acoustic lens transducer, ③ ultrasonic beam, ④ focal spot, ⑤ water bath, ⑥ tube reactor, ⑦ latex membrane, and ⑧ micelles.

sufficiently low to be acceptable by the human body. However, with high-frequency ultrasound, the cavitation becomes weak. This makes high-frequency ultrasound less effective in disrupting polymer micelles. For instance, Rapoport et al. found a very slow release of doxorubicin from micelles of Pluronic P105 under 1 MHz high-frequency ultrasound.[24] Therefore, it is of fundamental interest to develop block copolymer micelles that can be efficiently disrupted by high-frequency ultrasound, that is, under a relatively weak cavitation effect.

In this paper, we report on the finding that micelles formed by the amphiphilic diblock copolymer of poly(ethylene oxide)-*block*-poly(2-tetrahydropyranyl methacrylate) (PEO-*b*-PTHPMA) are sensitive to high-frequency ultrasound (1.1 MHz). The results suggest the occurrence of hydrolysis of THPMA side groups induced by the ultrasound irradiation, as depicted in Figure 1a. The use of PEO-*b*-PTHPMA was rationalized by the following consideration. The acoustic cavitation results in the formation, growth, and collapse of micrometer-sized bubbles in solution, while the collapse of bubbles could generate high temperature and pressure within the nanosecond scale and a solvodynamic shear effect.[28] To make a block copolymer micelle react to a relatively weak cavitation effect, the polymer should contain weak chemical bonds, ideally mechano-labile and thermo-labile bonds that are sensitive to the mechanical and thermal effects associated with the cavitation. A previous study on the PEO-*b*-PTHPMA micelles in aqueous solution found that the hydrolysis of THPMA not only is sensitive to acid pH but could also be activated by thermal effect.[29] Upon hydrolysis, PEO-*b*-PTHPMA micelles are disrupted by an increased polarity over the conversion of PTHPMA to poly(methacrylic acid) (PMAA) (Figure 1). Indeed, as shown below, this study found that the hydrolysis of THPMA could be induced when the micellar solution was exposed to a high-frequency ultrasound at room temperature leading to the disruption of micelles. In line with the growing interest of exploring ultrasound to control chemical reactions,[30,31] this finding is important because it demonstrates a new way to develop ultrasound-sensitive block copolymer micelles based on ultrasound-triggered chemical reactions involving labile chemical bonds. We note that the two constituting polymers of PEO-*b*-PTHPMA are relevant to biomedical applications.[29,32]

2. Experimental Section

2.1. Preparation of PEO-*b*-PTHPMA Micelles. The synthesis of PEO-*b*-PTHPMA using atom transfer radical polymerization (ATRP) was previously reported.[29] The sample used in the present study was PEO_{112}-*b*-$PTHPMA_{164}$ (composition determined by ^1H NMR). Micelles were prepared by following the same procedure as previously reported.[29] Typically, 1.3 mg of PEO-*b*-PTHPMA was first dissolved in 5 mL of tetrahydrofuran (THF), and then 5 mL of water was added slowly (about 30 μL for every 30 s) to induce the formation of micelles. The solution was stirred for 30 min before the addition of 40 mL of water. Finally, THF was removed by evaporation at 45 °C for 24 h. The initial polymer concentration was 0.03 mg/mL. For micelles loaded with Nile Red (NR, from Aldrich), the hydrophobic chromophore was dissolved in THF with the block copolymer (at a concentration of 0.04 mg/mL) before the addition of water and the evaporation of the organic solvent. Unloaded NR was precipitated in aqueous solution and removed by filtration through 0.45 μm membrane.

2.2. Exposure of Micellar Solution to High-Frequency Ultrasound Irradiation. The equipment employed in this study is a high-frequency focused ultrasound generator fabricated in-house. A schematic diagram of the apparatus is depicted in Figure 1b. It comprises two main components: an ultrasound generator and an acoustic lens transducer. The acoustic lens transducer with an effective diameter of 39 mm and a focal length of ~ 90 mm was mounted at the bottom of a tank filled with water, and the beams of ultrasound were pointed upward and focused on a circular spot with a diameter of about 3 mm. The ultrasound output power can be adjusted in the range of 0−200 W, and the frequency of ultrasound is 1.1 MHz. The focused beams of ultrasound can penetrate through a latex membrane and act on the PEO-*b*-PTHPMA micellar solution placed in a tube reactor. The volume of micellar solution in the tube reactor was 5−10 mL. The effects of ultrasound irradiation time, location of focal spot, and power output on the disruption behavior of micelles were investigated.

2.3. Characterizations. The pH value of the micellar solution upon ultrasound irradiation was measured by using a pH-meter (FE20-FiveEasy, Mettler Toledo). First, three buffer solutions with different pH values (4.003, 6.864, 9.182) were used to calibrate the pH-meter. Then the electrode probe was inserted into the micelle solution before high-intensity focused ultrasound (HIFU) irradiation and after HIFU irradiation for different times. Infrared spectra were recorded at room temperature on a Nicolet 560 Fourier transform infrared (FTIR) spectrometer. For those measurements, thin films of the micelles exposed to ultrasound irradiation were prepared as follows. After the micellar solution at a PEO-*b*-PTHPMA concentration of 1.36 mg/mL was subjected to ultrasound for a certain time, the solution was dried under vacuum at 60 °C until water was completely removed to obtain the dry sample. The dried sample was then redissolved in THF, cast on a KBr window, and dried. Dynamic light scattering (DLS) was performed on a Brookhaven BI-200 goniometer with vertically polarized incident light of wavelength λ = 532 nm supplied by an argon laser operating at 200 mW, and a Brookhaven BI-9000 digital

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autocorrelator. Measurements were made at 25 °C and at an angle of 90°. The autocorrelation functions from DLS were analyzed by using the non-negatively constrained least-squares algorithm to obtain the distribution of hydrodynamic diameters. Before the DLS measurement, the micellar solution was filtered through a 0.45 μm membrane. The morphology of the micelles was observed by atomic force microscopy (AFM, NanoScope MultiMode IIIa). The samples were prepared by casting a drop of the micellar solution on clean mica surface followed by drying. Fluorescence emission spectra were recorded on a Hitachi F-4500 double-monochromator spectrophotometer, with the excitation wavelength being 550 nm.

3. Results and Discussion

As mentioned above, with high-frequency, high intensity focused ultrasound (HIFU) can be obtained. A high power ultrasound beam is brought to a tight focus at a distance from the transducer.[33,34] HIFU has a much stronger interaction with the substances in the focal spot than in other locations and can produce both thermal and nonthermal effects that are useful for biomedical treatment and sonochemical reaction. When an ultrasonic wave passes through a liquid medium, a large number of microbubbles form, grow, and collapse in a very short time (about a few microseconds), which is called ultrasonic cavitation. Theoretical calculations and experiments suggested that ultrasonic cavitation can generate a local temperature as high as 5000 K, a local pressure as high as 500 atm, and heating and cooling rates greater than 10^9 K/s.[28] Under such vigorous conditions, the decomposition of solvent or monomer, or rupture of polymer chains can take place.[35,36] Although the cavitation effect is weakened with increasing the ultrasound frequency,[37] it cannot be neglected under a high ultrasonic intensity especially under a focused mode. Some reports showed that ultrasonic cavitation indeed could occur under HIFU.[38,39] On the basis of this analysis, it was reasonable to assume that high-frequency ultrasound could affect polymer micelles in aqueous solution, and that the effect could be significant for polymers that contain labile chemical bonds such as the acetal groups in PEO-*b*-PTHPMA micelles.

Indeed, we found evidence that focused high-frequency ultrasound could disrupt PEO-*b*-PTHPMA micelles as a result of ultrasound-triggered hydrolysis of THPMA groups. The most clear indication is the decrease in the pH value of the micellar solution upon ultrasound irradiation, which suggests the conversion of THPMA to MAA (acid) groups due to the hydrolysis-induced cleavage of tetrahydropyran-2-ol (Figure 1a). Figure 2 shows the variation of pH value as a function of the time of ultrasound irradiation (output power, 200 W) by placing the focal spot of the beam at three locations. With the focal spot in the middle of the micellar solution, the effect appeared to be the most important. However, even with the solution moved above or below the focal point, the results show that the ultrasound waves around the focal spot remain very effective (the solution received the same ultrasound power, and the change in the focal spot location changed the wave intensity profile propagating through the solution). In all cases, the pH decreased significantly within the first hour. In the control experiments, no ultrasound was applied to the micellar solution under stirring at 25 °C, and the pH

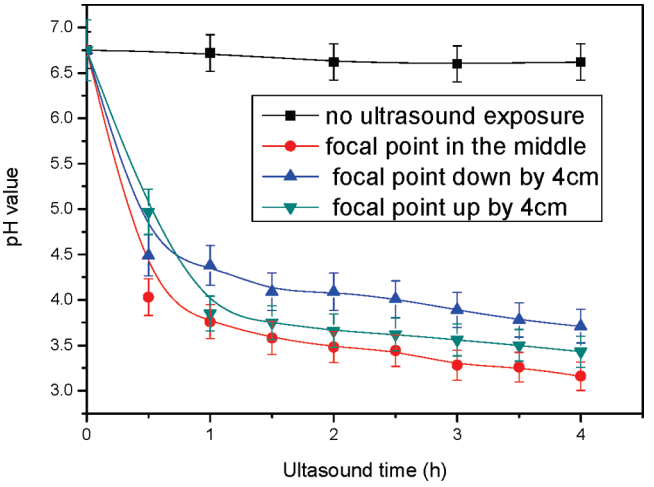

Figure 2. Change in pH of the micellar solution as a function of ultrasound irradiation time. By repositioning the tube reactor, the location of the focal point of the ultrasound beams can be varied (volume of micellar solution, 5 mL; ultrasound power, 200 W).

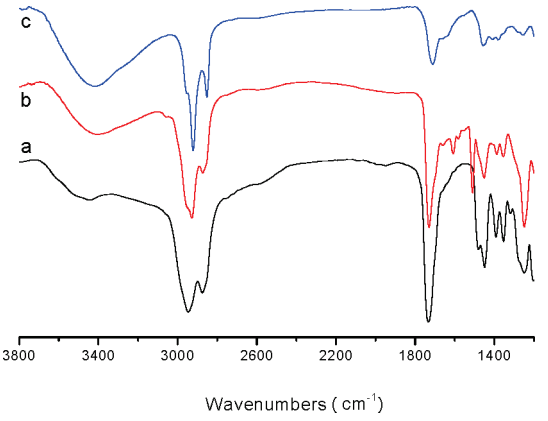

Figure 3. FTIR spectra of solid samples recovered from the PEO-*b*-PTHPMA micellar solution before (a) and after ultrasound irradiation for (b) 1 h and (c) 2 h of irradiation time.

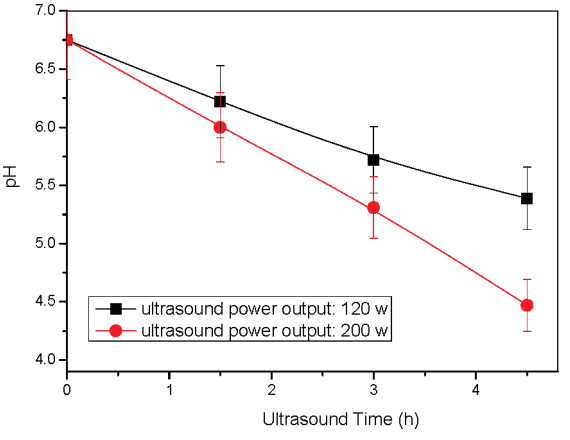

Figure 4. Change in pH as a function of ultrasound irradiation time for a PEO-*b*-PTHPMA micellar solution of 10 mL, using two different ultrasound power outputs.

value was nearly unchangeable after 4 h. Even by heating the solution to 90 °C, no significant decrease in pH was observed in the absence of ultrasound (data not shown). These results show that the decrease in the pH value of the micellar solution was

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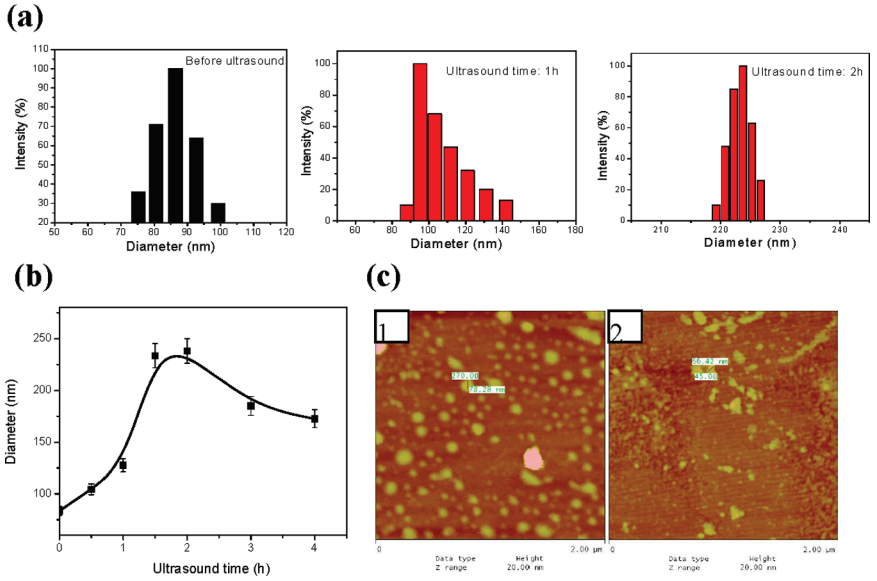

Figure 5. (a) DLS-revealed change in the size distribution of PEO-*b*-PTHPMA micelles in solution before and after ultrasound irradiation for different times. (b) Change in the apparent average hydrodynamic diameter of PEO-*b*-PTHPMA micelles with the ultrasound time. (c) AFM images of PEO-*b*-PTHPMA micelles before (c-1) and after 4 h of ultrasound irradiation (c-2).

caused by ultrasound and, without ruling out a thermal effect, suggest that the cleavage of THPMA could be mainly activated by the nonthermal (mechanical) effect produced by HIFU.

Infrared spectroscopic measurements provided additional evidence for the ultrasound-induced hydrolysis reaction in the micellar solution. Figure 3 shows the IR spectra recorded with dry samples collected from the micellar solution before and after ultrasound irradiation (1 and 2 h). Before HIFU, the ester groups in PEO-*b*-PTHPMA micelles displayed the characteristic carbonyl stretch band at ~1730 cm^{-1}. After 1 h of the ultrasound irradiation, the band appeared to be shifted due to a contribution from the carbonyl stretch mode of carboxylic acid groups. This spectral change became more prominent after 2 h of ultrasound irradiation, with the appearance of a "shoulder" band at ~1660 cm^{-1} corresponding to hydrogen-bonded carbonyl groups (acid dimers). Moreover, the more intense broad absorption band around 3400 cm^{-1} after ultrasound is indicative of the increasing amount of hydroxyl groups in the sample, from MAA and cleaved tetrahydropyran-2-ol molecules (Figure 1a). These results support the analysis that a hydrolysis reaction of PEO-*b*-PTHPMA micelles in aqueous solution could occur at room temperature under the ultrasound irradiation.

The disruption of polymer micelles by a focused ultrasound beam offers an appealing advantage over other stimuli for controlled delivery applications. In addition to the easy control over the time of triggering the reaction (temporal control), spatial control is possible since the ultrasound beams can be directed to the areas of interest. This is similar to photocontrollable polymer micelles.[12−14] However, using light, the penetration depth of photons is limited even with near-infrared light (generally in the range of millimeters). The setup in Figure 1 and the results in Figure 2 show that HIFU beams can easily go through long distances in an aqueous medium without attenuation of the disruption power. We performed more experiments by changing the volume of the micellar solution and the ultrasound output power, which are two variables that would influence the effect of the ultrasound irradiation on polymer micelles. Figure 4 shows the change in the pH value as a function of ultrasound time for a micellar solution of 10 mL, which is twice the volume used for the experiments in Figure 2. Two observations can be made. First, using the same ultrasound power of 200 W, the pH decrease is slower with the larger solution volume (10 mL versus 5 mL in Figure 2). Second, at the same volume of 10 mL, a lower ultrasound power (120 W) slows down the decrease in pH. These two observations have a logical explanation. Since the measured pH value reflects the degree of hydrolysis of PEO-*b*-PTHPMA micelles over the whole solution, an increased solution volume or a decreased ultrasound power should have an effect as a reduction of the rate of the ultrasound-induced reaction.

The above results suggest that the THP pendant groups in the PTHPMA block can be cleaved from the copolymer chain under HIFU at room temperature. As a result, the hydrophilic−hydrophobic balance of the micelle should be shifted to an increased hydrophilicity and thus lead to the disruption of PEO-*b*-PTHPMA micellar aggregates. The results of DLS and AFM measurements are shown in Figure 5 confirming the ultrasound-induced disruption of the polymer micelles. Upon ultrasound irradiation, the size distribution of micellar aggregates in solution changed with time, apparently shifting to larger aggregates, accompanied by an increase in polydispersity (Figure 5a). While the mean hydrodynamic diameter of the micelles was ~85 nm before HIFU, it increased under the effect of HIFU within the first 2 h, before decreasing at longer ultrasound times (Figure 5b). One possibility for the apparent size increase would be swelling of the polymer micelles as a result of the hydrolysis reaction leading to a more hydrophilic micelle core that could absorb more water molecules. However, ultrasound is known to be able to destroy original aggregates and form new aggregates over the course of disruption.[40] Moreover, in the present case of PEO-*b*-PTHPMA, with the conversion of THPMA to MAA, complexes could form due to H-bonding between PEO and MAA units at pH < 4.5.[41] An increase in the scattering intensity of the micellar solution after ultrasound exposure was observed, which seemed to support the second scenario; that is, larger aggregates could be formed after the ultrasound-induced disruption of the micelles. Even though AFM images cannot reflect the real status of aggregates in

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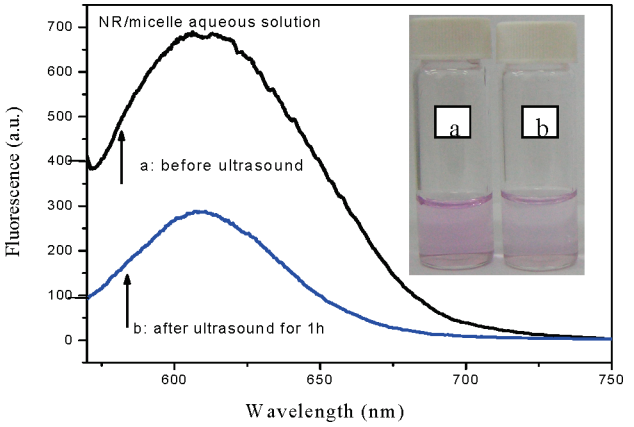

Figure 6. Fluorescence emission spectra (λ_{ex} = 550 nm) of NR/PEO-*b*-PTHPMA micellar solution before and after ultrasound at different times. Reaction volume of micelle solution: 5 mL. Inset: Digital pictures of NR/PEO-*b*-PTHPMA micelle solutions after filtering, (a) before ultrasound, and (b) after ultrasound for 1 h.

solution as DLS, since condensation or coalescence of aggregates could occur during the evaporation of water, the AFM images in Figure 5c show the morphological change of the micelles caused by ultrasound irradiation. While mostly spherical micelles were observed for the solution before HIFU (large aggregates are likely caused by condensation), after 4 h of ultrasound treatment of the solution larger aggregates with irregular forms appeared.

Finally, the release behavior of the hydrophobic compound Nile Red (NR) as a payload model from the PEO-*b*-PTHPMA micelles was investigated. The fluorescence emission of NR is known to be sensitive to the environment, being generally intense in a hydrophobic medium where NR can be solubilized, and much quenched due to aggregation in water where NR is insoluble.[13,42] Figure 6 shows the fluorescence emission spectra of a NR-loaded PEO-*b*-PTHPMA micellar solution before and after 1 h of ultrasound irradiation (5 mL; power output, 200 W). After HIFU, the emission intensity of NR was reduced by more than half of the initial value. This suggests the release of part of NR molecules into the aqueous medium. The effect can also be noticed from the photos in the inset. The pink color, which arises from the absorption of NR solubilized by the micelle cores, was partially faded after the ultrasound exposure, and the color of the solution became lighter. The apparently limited quenching of NR fluorescence agrees with the observation that micellar aggregates remained after HIFU, so that a significant portion of NR molecules remained solubilized. Another possible reason for this incomplete fluorescence quenching is that NR had a greater solubility in water at the acidic pH resulting from the ultrasound-induced hydrolysis of PEO-*b*-PTHPMA micelles. It is worth emphasizing that the release of NR upon ultrasound treatment was not reversible. After the HIFU was turned off after 1 h, the color of the solution could not be recovered and neither could the fluorescence intensity of NR. This indicates the absence of any re-encapsulation of NR and supports the conclusion that the disruption of PEO-*b*-PTHPMA micelles originated from a chemical reaction induced by HIFU, rather than physically changing aggregation states under the effect of ultrasound waves. This is different from the ultrasound-controlled, physically reversible release process with the Pluronic micelle/doxorubicin system developed by Rapoport et al.[24]

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4. Conclusion

PEO-*b*-PTHPMA micelles in aqueous solution respond to high-frequency HIFU irradiation. The decrease in pH of the micellar solution upon HIFU suggests that the ultrasound-generated, combined thermal and mechanical effect could induce the hydrolysis reaction of the labile THP acetal at room temperature, which converts THPMA to MAA. This conclusion was supported by infrared analysis. The irreversible disruption of PEO-*b*-PTHPMA micelles as a result of the HIFU-induced chemical reaction was evidenced by DLS, AFM, and fluorescence measurements. By adjusting the HIFU time, intensity, and location, the micelle disruption process could be tuned. This study shows the possibility of rationally designing ultrasound-sensitive block copolymer micelles by having labile chemical linkages in their structures that are likely to react to the effect of HIFU and create a shift of the hydrophilic−hydrophobic balance toward the destabilization of the micelles. As a remote noninvasive stimulus, the use of HIFU is attractive for controlled drug delivery applications. With HIFU-sensitive polymer micelles, ultrasound offers the desired temporal and spatial control over the disruption of drug carriers and the resulting release. This is similar to the use of light to trigger the disruption of photosensitive polymer micelles. However, unlike light, whose penetration depth is very limited, HIFU can act deep in aqueous solution. More studies are needed to disclose block copolymer chemical structures that can react more sensitively to HIFU beams in a controlled way.

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