High intensity focused ultrasound-responsive release behavior of PLA-b-PEG copolymer micelles

Hongji Zhang, Hesheng Xia*, Jie Wang, Yongwen Li
State Key Laboratory of Polymer Materials Engineering, Polymer Research Institute, Sichuan University, Chengdu 610065, China

Abstract
Poly(lactic acid) (PLA) was synthesized by solution polycondensation of L-lactic acid and further reacted with dihydroxyethyl poly(ethylene glycol) (PEG) to obtain the amphiphilic block copolymer PLA-b-PEG. The biodegradable PLA-b-PEG copolymer can self-assemble into spherical micelles in aqueous solution. Nile Red, as a payload model, was used to examine the release behavior of the micelles. The hydrophobic Nile Red can be adsorbed into the hydrophobic inner core of PLA-b-PEG micelles. With the introduction of Nile Red, the size of micelles increased. Moreover, high intensity focused ultrasound (HIFU), as a non-contact and remote control approach, was introduced to control the release behavior of PLA-b-PEG micelles containing Nile Red. The release behavior of Nile Red was monitored by fluorescence emission spectra. The results showed that HIFU can trigger the release of the encapsulated Nile Red from PLA-b-PEG micelles. By adjusting the HIFU time, intensity and location, the release behavior of Nile Red from micelles can be tuned. Based on the results, an irreversible release mechanism under HIFU was proposed. The irreversible release of Nile Red from the PLA-b-PEG micelle was attributed to a chemically breaking process of micelle structure due to the degradation of the PLA-b-PEG chain that resulted from the transient cavitation in the HIFU focal spot.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Amphiphilic copolymer micelle is one of most important kinds of drug carriers, because it can encapsulate many hydrophobic drugs such as doxorubicin (DOX), paclitaxel, and camptothecin, which are difficult to dissolve in aqueous solution directly. Among the amphiphilic copolymers, poly (lactic acid)-b-poly(ethylene glycol) (PLA-b-PEG) has attracted much attention for drug delivery, gene therapy, and tissue engineering due to its biodegradability, biocompatibility and proper hydrophilicity [1-3]. The degradation products from PLA-b-PEG can be excreted by the kidney or enter the Krebs cycle and thus are non toxic [3]. PLA/PEG block copolymers were obtained via trans-esterification with PLA [4] or ring-opening polymerization of (D, L)-lactide with poly(ethylene glycol) as the macroinitiator and stannous octoate Sn(Oct)2 as the catalyst [5,6].

On the other hand, for drug delivery, the controlled release of therapeutic compounds is of great interest, since some particular drugs are often restricted to a narrow dosing range and may also be restricted to a particular target tissue [7]. An optimal design of controlled release of therapeutic compounds is expected to accumulate intact at the disease site and then have the drug released locally at a controlled fashion. This can be achieved, at a certain extent, by chemical or physical means [8,9]. Up to now, light [10,11], temperature [12-15], pH [16,17], electric and magnetic field [18-20], and ultrasound [21-26] have been employed to control the release of drug from the copolymer micelles.

Ultrasound is a powerful physical modality for spatial and temporal control of on-demand drug delivery [27-31]. Ultrasound can effectively penetrate deep into the interior of the body in a non-invasive way. In general, ultrasound technology includes low frequency ultrasound and high frequency diagnostic ultrasound. For low frequency ultrasound, the ultrasound with a longer wavelength is difficult to focus and thus when low frequency ultrasound passes through the human body, the ultrasonic cavitation can destroy the vital structures. This restricts practical clinical use of low frequency ultrasound to some extent. For high frequency ultrasound, the ultrasonic wave can be focused, that means, the intensity is quite high only in the focal spot, while in other areas, the intensity can be low to be acceptable by human body. However, under the high frequency ultrasound, the cavitation becomes weak, and thus will have a negative effect on the release behavior. For example, no release of DOX was found in the Pluronic P105 micelle under the 500 KHz high frequency ultrasound [32]. Rapoport et al. reported a very slow release for Pluronic P105 micelle under 1 MHz high frequency ultrasound [25]. In order to improve the release efficiency for high frequency ultrasound, a focused ultrasound mode was needed. On the other hand, the release behavior under ultrasound closely relates to the micelle type. Some fast ultrasound-responsive micelle systems need to be developed.

In this study, the amphiphilic block copolymers PLA-b-PEG were prepared by a two-step method. High intensity focused ultrasound,
whose energy can be focused on a spot with a diameter of about 3 mm, was employed to control the release of the hydrophobic compound Nile Red as a payload model from the biodegradable PLA-b-PEG micelles. The effects of HIFU parameters and irradiation time on the release behavior of PLA-b-PEG micelles were investigated.

2. Materials and methods

2.1. Materials

L-lactic acid (85%–90%) and dihydroxyl poly(ethylene glycol) (PEG) with a molar mass of ~3500 were obtained from Tianjin Chemical Reagents Institute. Nile Red (NR), stannous octoate Sn(oct)2 and poly(ethylene glycol)-block-polypoly(propylene glycol)-block-poly (ethylene glycol) (PEO-PPO-PEO, Pluronic F-108) with an average molecular weight of ~14,600 and a PEO weight percentage of 82.5 wt.% were purchased from Aldrich. All other organic solvents were of analytic grade and used as received.

2.2. Synthesis of poly(L-lactic acid)

Poly(L-lactic acid) was synthesized by direct solution polycondensation of L-lactic acid, without the intermediate of lactide. The polycondensation was carried out by using a Soxhlet extractor packed with molecular sieves (4A) in the extractor cuvette. The experimental procedure is as follows: Typically, 50 ml L-lactic acid and 100 ml xylene were added to a 250 ml Soxhlet flask, while the reaction mixture was heated to 138 °C under stirring. The water was distilled, and then dropped through the 4A molecular sieves to be removed, and the distilled xylene went back to the reaction flask by a siphonic effect. The polymerization was carried out for 40 h, and the molecular sieves were replaced every 10 h. The product, after cooling, was dissolved in dichloromethane, and precipitated in the excessive anhydrous ethanol for 24 h at room temperature. The precipitate was filtered out and dried at 60 °C under vacuum.

2.3. Synthesis of poly(L-lactic acid)-block-poly(ethylene glycol)

The process of PLA-b-PEG synthesis was similar with the above-mentioned. Typically, 1.0 g PLA and 5.0 g PEG (five times excess) were introduced into a 250 ml Soxhlet flask, the polymerization was carried out for 30 h, while stannous octoate (0.1 wt.%, calculated on the amount of PLA) was used as the catalyst. All the reactions were carried out under the common atmospheric environment. The resultant block copolymer was dissolved in dichloromethane, and precipitated in the excessive anhydrous ethanol for 24 h at 3 °C. The precipitate was centrifugalized at 10,000 rpm and dried at 40 °C under vacuum.

2.4. Preparation of PLA-b-PEG micelles

The obtained PLA-b-PEG was firstly dissolved in the tetrahydrofuran (THF) by heating at 40 °C for 3 h. The copolymer micelle was formed by adding water to PLA-b-PEG/THF solution. Typically, 15 mg PLA-b-PEG was dissolved in 5 ml THF, and then 30 ml water was added dropwise overnight under vigorous stirring to induce the formation of the micelles while THF was allowed to evaporate by heating.

2.5. Preparation of PLA-b-PEG micelles containing Nile Red

Nile Red was firstly dissolved in THF with an initial concentration of 0.02 mg/ml, and then a predetermined amount of PLA-b-PEG was introduced to 5 ml NR/THF solution, followed by heating the solution at 40 °C for 3 h to ensure that the polymer dissolves completely. After cooling, 30 ml water was added dropwise to induce the formation of PLA-b-PEG micelles containing Nile Red, and the mixed solution was stirred for 24 h at room temperature while the THF was evaporated by heating.

As a control experiment, the preparation of PEO-PPO-PEO micelles containing Nile Red is similar to that of PLA-b-PEG micelle as mentioned above.

2.6. High intensity focused ultrasound-responsive release behavior of PLA-b-PEG micelle

The equipment employed in this research is a high intensity focused ultrasound generator which was made in-house. It comprises two main components: ultrasound generator and acoustic lens transducer. The schematic diagram of apparatus was shown in Fig. 1. 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 upwards 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 passed through latex membrane and acted on the PLA-b-PEG/NR micellar solution in the cuvette reactor. The effects of HIFU irradiation time, location and power output on the release behavior of micelles were investigated.

2.7. Characterization

Fourier transform infrared (FTIR) analysis of the samples was performed on a Nicolet 560 FTIR spectrometer. Proton nuclear magnetic resonance (1H NMR) spectra were recorded at room temperature with a Bruker spectrometer operating at 400 MHz by using deuterated chloroform (CDCl3) as a solvent and tetramethylsilane as an internal reference. The molecular weight was measured by gel permeation chromatography (GPC; Agilent 1100 Series) with tetrahydrofuran (THF) as the eluent at a flow rate of 1 ml/min at 35 °C. The molecular weights were calibrated with polystyrene standard. 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 AT digital 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 square algorithm (NNLS) method to obtain the diameter distributions. The micelle morphology was observed with transmission electron microscopy (TEM; JEM 100-CX), atomic force microscopy (AFM;
NanoScope MultiMode IIIa) and scanning electron microscope (SEM; Inspect F, Philips). Specimens of TEM were prepared by dipping a copper grid into aqueous solutions of copolymers at a concentration of 0.05 mg/ml and the grid was air dried before measurements. AFM samples were prepared by dropping the micellar solution onto the mica and then the sample was freeze dried. Specimens for SEM observations were prepared by depositing one drop of micellar solution onto silicon wafer and air dried before measurements. Steady-state fluorescence emission spectra of NR/PLA-b-PEG micelle solutions were recorded on a F-4500 (Hitachi, Japan) double-monochromator spectrophotometer. The excitation wavelength (λex) was 550 nm, and the EX Slit and EM Slit were 5.0 nm.

3. Results and discussion

3.1. Synthesis and characterization of PLA and PLA-b-PEG block copolymer

The PLA-b-PEG block copolymer was dominantly synthesized by ring-opening polymerization in the last decades. The ring-opening polymerization accompanies high cost due to the use of expensive lactide and uncharitable reaction condition. In this study, poly(lactic acid) (PLA) was synthesized by solution polycondensation of L-lactic acid and further reacted with dihydroxyl poly(ethylene glycol) (PEG) with the aid of Soxhlet extractor under a common atmospheric environment to obtain the amphiphilic block copolymer PLA-b-PEG. During the polycondensation, a key step is to remove the side products, i.e. water. In this study, the water was distilled during the reaction, and then condensed through the 4A molecular sieves packed in the Soxhlet extractor to be removed. This process is relatively easy and low-cost. The obtained PLA-b-PEG copolymer was characterized by GPC, FTIR and 1H NMR techniques (see Supplementary data Fig. S1–3). The number average molecular weight (Mn) and the weight average molecular weight (Mw) of the obtained PLA-b-PEG are ~7000 and ~10,000, respectively. Table 1 shows that the Mn of PLA-b-PEG is nearly equal to the sum of the Mn of block PEG and PLA. A single peak was observed in the GPC curves, indicating that the copolymer contained no PEG or PLA homopolymer. The strong peak appeared at ~2885 cm⁻¹ in the FTIR spectrum of PLA-b-PEG copolymer, which was attributed to the C–H stretching band of the PEG block. The absorption peak at ~1758 cm⁻¹ was characteristic of carbonyl (–C=O) in PLA block, and the peak at ~1096 cm⁻¹ was attributed to the stretching vibration of C–O–C in PEG block. Furthermore, the bands at ~951 and ~843 cm⁻¹ were characteristic of the crystalline phase of PEG [33], while the band at ~755 cm⁻¹ was assigned to the amorphous phase of PLA [34]. In the 1H NMR spectra of PLA and PLA-b-PEG block copolymer, the chemical shifts at ~5.17 ppm (a) and ~1.60 ppm (c) were assigned to methine (–CH) and methyl (–CH₃) protons in PLA blocks, respectively. Quartet split peaks of methine (5.13, 5.15, 5.17, 5.19 ppm) and doublet split peaks of methyl (1.57, 1.59 ppm) appeared in the 1H NMR spectra of PLA-b-PEG. The chemical shift at 3.64 ppm (singlet, b) was assigned to the methine (CH₂) protons in PEG blocks. All the results confirmed the structure of the obtained PLA-b-PEG block copolymer.

3.2. Preparation and characterization of PLA-b-PEG micelle

Based on the prepared PLA-b-PEG, the copolymer micelles were induced to form by adding water dropwise to PLA-b-PEG/THF solution. The size and morphology of the PLA-b-PEG micelle were characterized by DLS, TEM and AFM. DLS was employed to evaluate the size and size distribution of PLA-b-PEG and NR/PLA-b-PEG micelles. Fig. 2 shows the size and size distribution of PLA-b-PEG micelles in aqueous solutions at different concentrations.

As shown in Fig. 2a, b, and c, the mean diameters of PLA-b-PEG micelles are ~70 nm, ~80 nm, and ~90 nm at the PLA-b-PEG concentrations of 0.05 mg/ml, 0.2 mg/ml, and 0.5 mg/ml, respectively. The size of micelles increased with increasing copolymer concentration, which suggests that the micelle size not only relates to the length of hydrophobic block, but also the concentration of amphiphilic polymer. Additionally, the mean diameter of micelles with entrapped NR became bigger compared to the blank micelles. The mean diameter of the NR/PLA-b-PEG micelle is ~125 nm at a PLA-b-PEG concentration of 0.05 mg/ml (Fig. 2d). Also, it can be noted that the size distribution became wider compared to the micelles without NR. This may be caused by the different content of NR encapsulated into the core of the different PLA-b-PEG micelles.

AFM was used to observe the size and morphology of micelles. Fig. 3 shows the AFM images of micelles with and without NR. In both cases the micelles were spherical. The mean diameter of micelles without NR observed by AFM was ~34 ± 6 nm and the size distribution was relatively uniform. Compared with blank micelles, the size of the micelles with NR increased to ~49 ± 9 nm and the size distribution became wider. The diameter of micelles observed by AFM is smaller than that obtained from DLS experiment. The reason is that the diameter of micelles obtained from DLS experiment reflects the hydrodynamic diameter of micelles that are swelled by water molecules, whereas the diameter of the micelles observed by AFM shows that of dried nanoparticles.

The micelle size and morphology were also observed by TEM (see Supplementary data Fig. S4). The results show that the morphology of PLA-b-PEG micelles is homogeneously spherical and the average diameter is about ~26 ± 5 nm, which is consistent with AFM results.

3.3. The encapsulation of Nile Red in the PLA-b-PEG micelle

Nile Red, as a model payload, is an important substance for the investigation on the controlled release behavior, particularly for the dilute micelle solution [35,36]. The blank micelle solution, water/NR mixed suspensions and micelles/NR mixed solution were prepared and deposited for 2 weeks, and then filtered. The colors for three samples were compared (see Supplementary data Fig. S5). The color of the blank PLA-b-PEG micelle is bluish-white due to the presence of nano-sized particles. The NR/water suspension is colorless, suggesting that the NR is not soluble in the water and can be removed after filtering. However, the color of NR/PLA-b-PEG micelle is pink, which suggests that in the presence of PLA-b-PEG, the hydrophobic dye NR can be entrapped into the hydrophobic core of PLA-b-PEG micelles and thus makes the micelle solution appear pink. To further characterize the encapsulation, or adsolubilization effect, the fluorescence experiments at an excitation wavelength of 550 nm were conducted (see Supplementary data Fig. S6). Like the blank micelle solution, the water/NR mixed solution has nearly no fluorescence emission at 500–700 nm due to a low solubility of NR in water. On the other hand, the micelle solution entrapped NR has a strong fluorescence emission peak at ~600 nm, suggesting that the hydrophobic NR is encapsulated, or adsolubilized into the hydrophobic core of PLA-b-PEG micelles.

3.4. Controlled release of Nile Red from PLA-b-PEG micelle by HIFU

When a hydrophobic compound like a drug is encapsulated into micelles, its release property will be the significant consideration. The release of NR can be monitored and revealed by the changes in their fluorescence emission spectra. When NR is released, i.e., enters
into the aqueous solution from the core of micelles, the fluorescence emission will be quenched by water molecules due to a very low solubility [10]. Due to its insolubility in the water, the released NR will be precipitated into the bottom of the cuvette reactor. The spontaneous release of Nile Red from PLA-b-PEG micelles in aqueous solution was examined (see Supplementary data Fig. S7). The fluorescence emission intensity was nearly kept unchangeable in 30 days at room temperature, which suggests that the spontaneous release rate of NR is much lower, in agreement with the previous reports [37,38]. At the same time, the spontaneous release of NR from PLA-b-PEG micelles without an external stimulation is not a controlled means.

Fig. 2. Size and size distribution of PLA-b-PEG micelles in aqueous solutions at the copolymer concentration of (a) 0.05 mg/ml, (b) 0.2 mg/ml, (c) 0.5 mg/ml and (d) PLA-b-PEG micelles containing Nile Red (0.05 mg/ml), determined by DLS.

Fig. 3. AFM images of (a) PLA-b-PEG blank micelles (1.0 µm × 1.0 µm), (b) PLA-b-PEG micelles containing Nile Red (1.3 µm × 1.3 µm) at the copolymer concentration of 0.05 mg/ml and (c) section analysis across the PLA-b-PEG /NR micelles.
In this study, as a controlled means, HIFU was introduced to promote the release of NR. The controlled release system was set up as shown in Fig. 1. HIFU was originally developed as an extracorporeal tool for the treatment of tumors [39]. The basic principle of HIFU is that with a spherically curved transducer or an array of transducers, a high power ultrasound beam can be brought to a tight focus at a distance from its source. HIFU has a much stronger interaction with the substance in the focal spot than in other locations away from the focal spot. HIFU can produce the thermal effect and non-thermal effect, especially by a remote and non-invasive means. Both effects are useful for biomedical treatment and sonochemical reaction. The non-thermal effect mainly resulted from the acoustic cavitation. HIFU has the potential to act as a safe and site-specific external trigger of drug delivery.

**Fig. 4** shows the fluorescence emission spectra of NR/PLA-b-PEG micelle after a 15 min HIFU irradiation in the different locations of ultrasonic bath at a power output of 200 W. The decrease in the fluorescence emission intensity in the focal spot (F point in Fig. 4) is much more significant than that in the other locations away from the focal spot (A, B and C points in Fig. 4), which indicates HIFU more easily promotes the breaking of micelle in the focal spot. Like the optical focusing, the ultrasonic beam near the focal point F is densest and the energy is strongest, while the ultrasonic beam is sparse in the location of A, B and C. Also it was found that the change in the fluorescence emission intensity is not reversible once the HIFU is off. The significant discrepancy in the release behavior at different locations suggests that the energy of high intensity focused ultrasound can be focused on a specific spot. The experiment indicates that HIFU has the ability to control the drug release at a specific disease site.

**Fig. 5(a)** shows the change in the fluorescence emission intensity of the micelle solution encapsulated NR with the HIFU irradiation time at a power output of 160 W. The reaction temperature is ~44 °C. The fluorescence emission intensity decreases with increasing irradiation time. The decrease in the fluorescence intensity, to a certain extent, is proportional to the amount of NR released. The percentage of released NR was evaluated using the following equation: \( \% \text{release} = \frac{I_0 - I_t}{I_0} \), where \( I_0 \) is the fluorescence emission peak intensity at ~600 nm recorded before ultrasound, \( I_t \) is the fluorescence emission peak intensity at ~600 nm recorded by exposing the aqueous solutions of PLA-b-PEG micelles encapsulated NR to HIFU for \( t \) min. The percentage of released NR vs. time was shown in **Fig. 5(b)**. It can be noted that the percentage of released NR increased with the irradiation time. The digital pictures of NR/PLA-b-PEG micelle solutions at different irradiation times were also shown in **Fig. 5(c)**. It is clear that as the irradiation time increased, the pink color of micelle solution became weak gradually. This provides direct evidence that NR encapsulated in the hydrophobic core of micelles was released into aqueous solution under HIFU irradiation.

**Fig. 5.** (a) Variation of fluorescence emission spectra of Nile Red/PLA-b-PEG micelle solutions (\( \lambda_{\text{ex}} = 550 \text{ nm} \)) with the HIFU time; (b) the calculated released percentage of Nile Red from PLA-b-PEG micelle with the HIFU time and (c) the digital pictures of Nile Red/PLA-b-PEG micelle solutions at different HIFU time, (A) 0 min, (B) 30 min and (C) 60 min.

![Fig. 4. Fluorescence emission spectra of Nile Red/PLA-b-PEG micelle solutions (\( \lambda_{\text{ex}} = 550 \text{ nm} \)) after a 15 min HIFU in the different location of ultrasonic bath: (F) positioned at the focal spot, (A,B,C) 6 cm left, right and front of focal spot in the focal plane.](image)

![Fig. 6. SEM images of PLA-b-PEG micelles before and after HIFU irradiation.](image)
But the micelle nanoparticles still can be observed, indicating that the Nile Red was not fully released during the irradiation, and this is consistent with the existence of the fluorescence emission peak after HIFU irradiation for 120 min as shown in Fig. 5(a).

Fig. 7 shows the changes in the fluorescence emission intensity (a) and the percentages of released NR (b) at the different HIFU power outputs after 60 min irradiation. The reaction temperature increased slightly with increasing HIFU power output. With increasing HIFU power output, the decrease in the fluorescence emission intensity became more pronounced and the percentage of released NR increased. At a lower HIFU power output of 40 W, the percentage of released NR is ~40%, while it reaches ~65% at a higher HIFU power output of 200 W. This should be attributed to a stronger cavitation effect at a higher acoustic intensity.

3.5. A proposed mechanism for the irreversible release of Nile Red from PLA-b-PEG micelle by HIFU

The above fluorescent emission spectra experiments confirmed that the Nile Red can be released from PLA-b-PEG micelle under HIFU, which means that HIFU can break the micelle structure. Some questions are addressed: why and how can HIFU break the micelle structure? Is this breaking process chemical or physical? And what is the interaction between the HIFU and micelle? As mentioned above, the release of Nile Red from the copolymer micelle depends on the HIFU intensity, the location of reactor cuvette, and the HIFU time. Also the release of Nile Red is irreversible, i.e. after HIFU is stopped, the Nile Red cannot return to the core of PLA-b-PEG micelle, which means that once the micelle was disrupted, the molecules cannot be re-organized to the original shape. This phenomenon indicates that the PLA-b-PEG molecules were degraded under HIFU. If the micelle is just physically disturbed by HIFU, the amphiphilic copolymer can be reassembled into the original micelle structure once the ultrasound is off. However, if the micelle is chemically broken resulting from the molecule decomposition, it can’t be re-formed. Based on those facts, a mechanism was proposed as shown in Fig. 8. Due to the hydrophobic interaction between the Nile Red and PLA block, Nile Red was encapsulated in the micelle core. When the micelle solution was subjected to the HIFU irradiation, PLA-b-PEG copolymer was degraded due to the ultrasonic cavitation and the amphiphilic structure of copolymer was broken, consequently, the micelle was disrupted and the encapsulated Nile Red was released.

The key evidence for the proposed mechanism was the ultrasonic degradation of PLA-b-PEG. It is necessary to determine the variation of...
molecular weight of PEG-b-PLA with the HIFU time. As shown in Fig. 9, the molecular weights of PEG-b-PLA copolymer decreased with the HIFU irradiation time, which suggests that the PEG-b-PLA copolymer was degraded after HIFU irradiation. Indeed, the exact mechanism of ultrasonic degradation still remains obscure, but it is well accepted that the large shear fields and instantaneous hot spot produced by ultrasonic cavitation are primarily responsible for the degradation of polymers [40, 41]. 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. Ultrasonic cavitation includes stable and inertial or transient cavitation. Sonochemical theoretical calculations and corresponding experiments suggested that ultrasonic inertial 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, a very vigorous environment [42]. Under such vigorous conditions, the decomposition of solvent, monomer, or rupture of polymer chains can take place [43]. Especially, at the stage of the collapse of the cavity, radiated friction forces and shock waves generate the stresses on the surface of a polymer chain and/or more possibly within the polymer coil, resulting in bond breaking of the macromolecular chain in the liquid, which is similar to hydrodynamic shear degradation [44]. The previous research suggests that ultrasonic depolymerization is a non-random process with the chain scission occurring preferentially at the midpoints of polymer chains and with larger molecules degrading the fastest [44]. Another unique feature is that ultrasonic degradation reduces the molecular weight simply by splitting the most susceptible chemical bond in the chain [45].

Although the ultrasonic cavitation effect weakened with increasing ultrasound frequency [44], cavitation cannot be neglected under a high ultrasonic intensity especially under a focused spot. A lot of research confirms that ultrasonic cavitation can take place under HIFU. Zhu et al. studied inertial cavitation activity of HIFU by using terephthalic acid as hydroxyl (OH) radical scavengers [46]. Thomas et al. used polyacrylamide phantoms to gain insight into the behavior of cavitation activity in the focal region of the HIFU transducer and suggested that cavitation is the source of a previously observed enhanced heating effect in HIFU [47].

Reich reported that at intensities of 40 W and above, a significant Mw-reduction for poly (lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) could be observed even after short exposure times of 30 or 20 s, respectively [48]. Wheatley et al. also observed the degradation of PLGA under a high frequency ultrasound of 5–10 MHz [49]. As for the PLA-b-PEG block copolymer, it is believed that the most susceptible chemical bond should be the ester bond, especially the linkage connecting PEG and PLA block where it endures the strain of phase separation. Also the linkage connecting PEG and PLA blocks was approximately located at the center of the polymer chain. This degradation was accelerated by the introduction of PEG block into PLA chain, because hydrophilic PEG can enhance the penetration of water and rate of hydrolysis compared to PLA homopolymer [50]. Therefore, ultrasonic degradation of PLA-b-PEG block copolymer should be attributed to the scission of the ester bond connecting PEG and PLA block. Consequently, the disruption of PLA-b-PEG micelles occurs because the amphiphilic structure of the polymer was destroyed, resulting in the irreversible release of NR from the core of PLA-b-PEG micelles into the aqueous solution.

To confirm the role of ultrasonic cavitation, the thermal effect of HIFU should be excluded. There are two effects for HIFU: thermal effect (high temperature) and non-thermal effect (ultrasonic cavitation). During the HIFU irradiation, the temperature of the sample in the hot spot will increase. In this study, the reactor cuvette was put in the water bath, and the temperature of the micelle solution was below 50 °C during HIFU. Those actual temperatures at different power outputs were shown in Fig. 7. The effect of temperature on the release of NR from PLA-b-PEG micelle was investigated without HIFU irradiation. The NR/PLA-b-PEG micelle solutions were heated for 60 min at various temperatures under a vigorous magnetic stirring and then the fluorescence emission spectra were measured. Fig. 10 shows that the fluorescence emission intensity was nearly kept unchangeable during heating for 60 min. And even at a relatively high temperature such as 95 °C, the release rate of NR is still very low without HIFU irradiation. Therefore, temperature is not responsible for the release of NR from the core of PLA-b-PEG micelle into the aqueous solution. The non-thermal effect, i.e. ultrasonic cavitation should be the main reason to cause the release of the Nile Red.

To justify the above release mechanism, we have to exclude another possibility, i.e. the degradation of NR. To make sure that the observed decrease in fluorescence emission of NR was not caused by the degradation of NR under HIFU, a control experiment for NR/THF solution was conducted. The NR was firstly dissolved in organic solvent THF and then was subjected to the HIFU treatment. The fluorescence emission spectra of NR in THF solution before and after HIFU irradiation were recorded (see Supplementary data Fig. S8). The results show that after HIFU irradiation for 60 min, no notable change was observed in the fluorescence emission spectra of NR, indicating that the degradation of NR didn’t occur under the used HIFU experimental conditions.

As we know, the factors affecting the release behavior in the present system include HIFU parameters (intensity, focal spot,
frequency and time), the type of entrapped substance, as well as the micelle (type, concentration). The micelle type is especially very important for HIFU responsive behavior. To confirm the above mechanism, we also investigated HIFU responsive behavior of the NR/PEO-PPO-PEO system. The results were shown in Fig. 11. After 120 min HIFU, the color of the micelle solution didn’t change. Interestingly, the fluorescent emission spectra increased slightly after HIFU irradiation for 120 min, which is totally different from that for PEG-b-PLA micelle. The NR was not released, instead, a better adsolubilized effect was observed under the effect of ultrasound dispersion. This result suggests that the block copolymer PEO-PPO-PEO without a labile bond in the chain has a different HIFU responsive behavior as PEG-b-PLA. For NR/PEO-PPO-PEO micelle system, the micelle disruption by HIFU is physical and thus the release and recapsulation of the NR from PEO-PPO-PEO micelle is reversible. This reversible process also was found in DOX/PEO-PPO-PEO system [51,52]. The results further suggested that the irreversible release of Nile Red from PEG-b-PLA block copolymer micelle was caused by the chemical disruption of PEG-b-PLA micelle. On the other hand, this result also suggested that the degradation of NR didn’t occur under the used HIFU experimental conditions.

4. Conclusion

Poly(lactic acid) (PLA) was synthesized by solution polycondensation of L-lactic acid and further reacted with dihydroxyl poly(ethylene glycol) (PEG) to obtain the amphiphilic block copolymer PLA-b-PEG. The PLA-b-PEG copolymer micelles were induced to form by adding water dropwise to PLA-b-PEG/THF solution. Due to the hydrophobic interaction the spherical micelles of PLA-b-PEG can entrap the hydrophobic substances like Nile Red into its hydrophobic inner core. High intensity focused ultrasound, as a non-contact and remote control approach, was used to control the release behavior of PLA-b-PEG micelles containing Nile Red. The results showed HIFU can trigger the release of the encapsulated Nile Red from the copolymer micelle. By adjusting the HIFU time, intensity, and location or reactor, the release behavior of Nile Red can be tuned. Based on the results, an irreversible release mechanism under HIFU was proposed. The irreversible release of Nile Red from PLA-b-PEG micelle was attributed to a chemically breaking process of micelle structure due to the degradation of PLA-b-PEG chain resulting from the transient cavitation in the HIFU focal spot. This study will open up a new way for HIFU-copolymer micelle drug delivery system.

Acknowledgement

This project was supported by the Program for New Century Excellent Talents in Universities, China, National Natural Science Foundation of China.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jconrel.2009.05.037.

References


Fig. 11. Variation of fluorescence emission spectra of Nile Red/PEO-PPO-PEO micelle solutions (λex = 550 nm) before and after HIFU irradiation for 120 min at a power output of 160 W. (Inset) the digital pictures of Nile Red/PEO-PPO-PEO micelle solutions before (left) and after HIFU (right).


