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Drug releasing behavior of hybrid micelles containing polypeptide triblock copolymer

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ABSTRACT

We report a new type of hybrid polymeric micelles for drug delivery applications. These micelles consist of PLGA (PLGA: poly(L-glutamic acid)) and PEG (PEG: polyethylene glycol) mixed corona chains. In acidic condition, PLGA undergoes a transformation from water-soluble random coils to water-insoluble α-helix, leading to microphase separation in micelle coronas and formation of PEG channels. These channels connect the inner core and the outer milieu, accelerating the diffusion of drugs from micelles. The micelles were prepared through a co-micellization of PLGA-*b*-PPO-*b*-PLGA (PPO: poly(propylene oxide)) and PEG-*b*-PPO in water. During the self-assembly, the PPO blocks of both block copolymers aggregated into cores that were surrounded by mixed corona chains of PLGA and PEG blocks. We confirmed this structure by using a number of characterization techniques including nuclear magnetic resonance spectroscopy, zeta potential, circular dichroism, and dynamic light scattering. We also performed molecular dynamics (MD) simulations to verify the models of the hybrid micelle structure. One advantage of the hybrid micelles as drug carriers is their tunable release rate without sacrificing colloidal stability. The rate can be tuned by either micelle structures such as the composition of the mixture or external parameters such as pH.

1. Introduction

Amphiphilic block copolymers are able to self-assemble in water into micelles at nanometer scale. All of the micelles have core–shell structures with water-insoluble blocks forming cores and water-soluble blocks acting as coronas. One promising application of this type of aggregates is for drug delivery [1–9]. Usually, hydrophobic drugs can be loaded in the cores of the micelles to lower its toxicity in human body, and to prolong their circulation time in blood [10]. In addition, the nanostructure of micelles may help the aggregates penetrate through cell membrane to deliver drug at subcellular level [11–19].

Because most pathological processes exhibit a decrease in pH [20], it has become an interesting research topic to design micellar carriers with pH-regulated releasing rate, but without loosing colloidal stability. Contrary to the normal blood pH of 7.4, the extracellular pH values in tumorous tissues are determined to be around 6.5–7.0, and the intracellular environment is typically acidic (pH 5.0–6.0). Therefore, if a carrier can retain the drug at pH 7.4 and quickly release it at a relative lower pH will be ideal for clinical treatments.

Although pH-responsive block copolymer micelles have been extensively studied and suggested for the application of drug delivery [21–23], it is still highly demanded to design carriers which can vary drug releasing rate within a narrow pH window from 7.4 to 5.0 without loosing the integration of the particles [18]. Recent researches into hybrid micelles self-assembled from two block copolymers provide an opportunity for the design of this type of aggregates [24–31]. Jerome et al. prepared water-soluble micellar complexes of P2VP-*b*-PEG (P2VP: poly(2-vinylpyridinium); PEG: poly(ethylene oxide)) and PMAA-*b*-PEG (PMAA: polymethacrylic acid) [30]. This hybrid micelle consists of an interpolyelectrolyte complex core formed by the association of the PMAA and P2VP blocks surrounded by a corona of PEG blocks. Shi et al. prepared complex micelles from the self-assembly of P*t*BA-*b*-PNIPAM (P*t*BA: poly(*tert*butyl acrylate), PNIPAM: poly(*N*-isopropylacrylamide)) and P*t*BA-*b*-P4VP (P4VP: poly(4-vinylpyridine)) [31]. The hydrophobic P*t*BA blocks of the two polymers associated together to form

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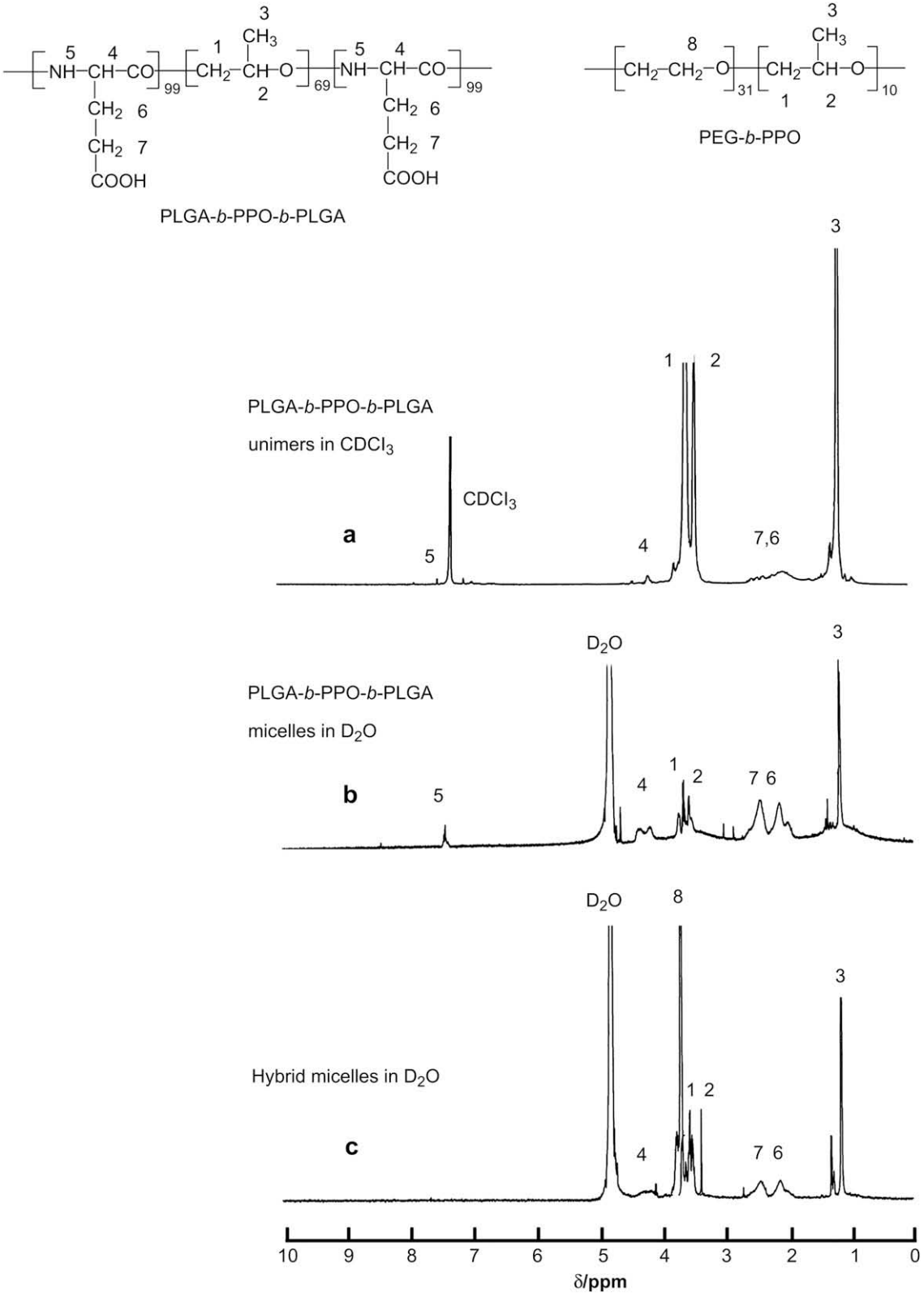


Fig. 1. ^{1}H NMR spectra for (a) PLGA-*b*-PPO-*b*-PLGA in $CDCl_3$, (b) in D_2O, and (c) PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO hybrid micelles with mixture ratio of 5/5 by weight in D_2O.

a dense core protected by the mixed P4VP/PNIPAM corona chains. These micelles with mixed corona chains are interesting and have a wide range of structure variation, because the solubility of P4VP and PNIPAM is sensitive to pH and temperature, respectively.

The new features of hybrid micelles offer the flexibility for further manipulating the desirable functions of drug carriers, especially releasing profile [32–35]. For the hybrid micelles with mixed corona chains, it is especially interesting to understand how

these mixed corona blocks influence drug release, whether the mixed chains in the corona can provide an advantage for the manipulation of releasing rate that micelles with single type of corona chains cannot afford. Unfortunately, there barely are reports to answer these questions.

In this paper, we report a new type of hybrid micelles self-assembled from PLGA-*b*-PPO-*b*-PLGA (PLGA: poly(L-glutamic acid); PPO: poly(propylene oxide)) and PEG-*b*-PPO in water to form micelles with mixed corona chains of PLGA and PEG. We are interested in this system for several reasons: (1) all the polymer blocks in the system have good biocompatibility and low toxicity which have potential for clinical treatments. (2) PLGA is a polypeptide segment which possesses a conformation transformation between coil and helix depending on pH of the solution, which has shown significant advantages in controlling both the function and supramolecular structures of bio-inspired self-assemblies [36–40]. (3) It is especially interesting to investigate how the conformation change of PLGA in the mixed corona chains can be used to manipulate drug releasing profiles of drug carriers.

The hybrid micelles were characterized by ^{1}H NMR, zeta potential measurements and dynamic light scattering (DLS). In addition to the experimental studies, we also performed molecular dynamics (MD) simulations using a Brownian dynamics to further verify the structures. The MD simulations offered a microscopic understanding of the thermodynamic properties and a detailed picture of the self-assembled aggregates [41–45]. Following the micelle characterization, we studied their behavior as drug carriers. Particularly we investigated the effect of mixed corona chains of PLGA and PEG on the drug releasing functions.

2. Experimental

2.1. Materials

Tetrahydrofuran (THF), hexane and 1,4-dioxane were refluxed with sodium and distilled immediately before use. Doxorubicin hydrochloride (DOX-HCl) was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd. α-Amino-ω-amino poly (propylene oxide) (NH_2–PPO_{69}–NH_2) and poly(ethylene glycol)$_{31}$-*b*-poly(propylene oxide)$_{10}$ (PEG-*b*-PPO) were purchased from Sigma–Aldrich Co., Inc. NH_2–PPO–NH_2 was dissolved in toluene in a flame-dried reaction bottle, followed by removing the toluene in high vacuum to obtain the initiator used for copolymerization. Cellulose membrane dialysis bags (1000 molecular weight cut-off) were provided by Serva Electrophoresis GmbH. All other solvents are of analytical grade and were used without farther purification.

2.2. Polymer synthesis and characterization

Poly(γ-benzyl-L-glutamate)-*b*-poly(propylene oxide)-*b*-poly(γ-benzyl-L-glutamate) (PBLG-*b*-PPO-*b*-PBLG) triblock copolymers were synthesized by ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) initiated by NH_2–PPO–NH_2 [46–48]. NH_2–PPO–NH_2 (0.2 g; 0.05 mmol) and BLG-NCA (2.7 g; 10 mmol) was dissolved in dioxane separately in two flame-dried reaction bottles, then, BLG-NCA solution was added to the solution of NH_2–PPO–NH_2 via a transfer cannula. The reaction was performed at 15 °C under a dry nitrogen atmosphere. After 72 h stirring, the reaction mixture became a viscous liquid and was poured into a large volume of anhydrous ethanol. The precipitated product was collected and dried under vacuum. The products were purified twice by repeated cycles of dissolution (in chloroform) and precipitation (in anhydrous methanol) of the products. PLGA-*b*-PPO-*b*-PLGA triblock copolymers were prepared by hydrolyzation of PBLG-*b*-PPO-*b*-PBLG with potassium hydroxide (KOH) [49]. As a brief, 1 g PBLG-*b*-PPO-*b*-PBLG was dissolved in 40 mL THF. In a separate step, an aqueous solution of KOH (3 mol equivalence with respect to benzyl group) was prepared and added to the solution of the polymers. After 4 h stirring, the mixture was acidulated with excessive HCl, and dialyzed in distilled water for about 72 h to remove organic solvents and other small impurities. The products were finally freeze-dried to get PLGA-*b*-PPO-*b*-PLGA powder.

The molecular weight of the block copolymer was estimated using ^{1}H NMR measurements (Avance 550, Bruker). Since the degree of polymerization (DP) of the PPO block is known (69), the total molecular weight of the triblock copolymer PBLG-*b*-PPO-*b*-PBLG can be calculated by the peak intensities of the methylene proton signal (5.1 ppm) of PBLG and the methylene proton signal (3.6 ppm) of PPO in the ^{1}H NMR spectrum. The calculation shows the molecular weight of the original copolymer is 47,300. The disappearance of methylene proton peak (5.1 ppm) of PBLG segments (see Fig. 1a) provides the evidence of deprotection of benzyl group from PBLG-*b*-PPO-*b*-PBLG copolymer to form PLGA-*b*-PPO-*b*-PLGA copolymer. The molecular weight of PLGA-*b*-PPO-*b*-PLGA is calculated to be 29,500 after hydrolysis. Accordingly, the degrees of polymerization of PLGA and PPO blocks are 99 and 69.

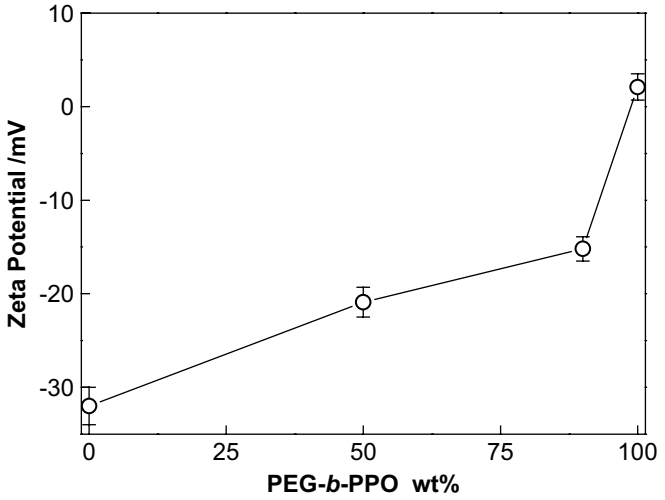


Fig. 2. Zeta potential of PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO micelle solutions as function of PEG-*b*-PPO content at pH = 7.4.

2.3. Preparation of hybrid micelles with and without drug-loaded

The mixtures of PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO with various weight ratios were dissolved in mixed solvent of *N*,*N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) (v/v = 4/1). The concentration is 0.6 mg/mL regardless of the ratio PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO. Distilled water was then added to the polymer solution at a rate of 1 drop every 5–10 s with vigorous stirring until water content is up to 25% of the original solution by volume. The mixed solution was dialyzed against distilled water for 72 h using a dialysis bag to remove organic solvents. The distilled water was exchanged at an interval of 3–4 h. The resulting solutions were adjusted to the desired concentration and pH values using hydrochloric acid (HCl) or KOH solution.

The preparation procedure of DOX-loaded hybrid micelles was similar to that of the hybrid micelles without drug-loaded. DOX-HCl and PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO mixtures with various weight ratio were first dissolved in DMF/DMSO mixed solvent (v/v = 4/1). The concentrations of DOX and copolymer mixtures were 0.3 mg/mL and 0.6 mg/mL, respectively. Triethylamine was added to the solution to remove hydrochloride and the distilled water was added to form pre-structure micelle. After stirring at room temperature overnight, the mixed solution was dialyzed against distilled water for 72 h at 25 °C. The distilled water was replaced every 3–4 h.

2.4. Laser light scattering measurements

Light scattering was measured by a LLS spectrometer (CGS-5022, ALV) equipped with an ALV-High QE APD detector and an ALV-5000 digital correlator using an He–Ne laser (the wavelength $\lambda = 633$ nm) as light source. The measurements were carried out at 20 °C or 37 °C. For the dynamic LLS measurements, the Laplace inversion of a measured intensity–intensity time correlation function $G^{(2)}(t,q)$ in the self-beating mode could result in a line-width distribution $G(\Gamma)$, where $q = 4\pi n \sin(\theta/2)/\lambda$ is the scattering vector as a function of scattering angle θ, n is the refractive index of the solution, and λ is the wavelength of the incident beam. The translational diffusion coefficient D calculated from the decay rate, Γ, by the slope of the Γ vs. q^2 plot, can lead to hydrodynamic radius R_h by the Stokes–Einstein equation $R_h = k_B T/(6\pi\eta D)$, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the solvent.

Table 1
R_h of hybrid micelles formed by mixtures of PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO with weight ratios of 8/2 and 2/8 at various temperatures and pH values

PLGA-*b*-PPO-*b*-PLGA/ PEG-*b*-PPO (weight ratio)	pH	7.4	5.5	4.0
8/2	R_h [nm] (37 °C)	47.7	43.3	39.6
	R_h [nm] (20 °C)	61.4	60.1	59.8
2/8	R_h [nm] (37 °C)	69.5	64.8	54.0
	R_h [nm] (20 °C)	81.4	75.0	67.2

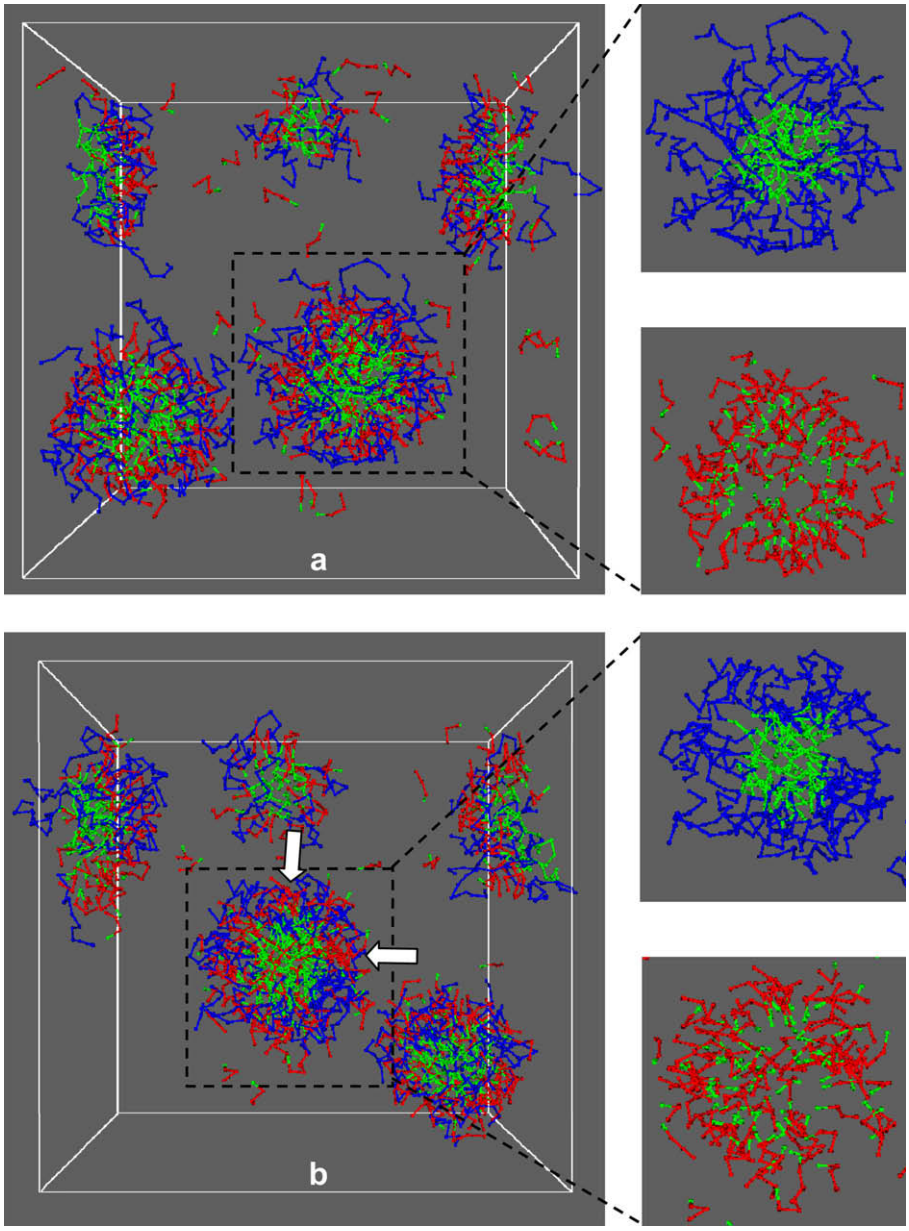


Fig. 3. Typical snapshots of $A_{10}B_7A_{10}$ triblock copolymer and B_1C_3 diblock copolymer systems in (a) basic and neutral condition and (b) acidic condition. The red, blue, and green lines represent C, A, and B blocks, respectively. Arrows point out the C block aggregation areas (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.

2.5. Zeta potential measurements of hybrid micelles without drug-loaded

A Doppler microelectrophoresis (Zetasizer 3000HS, Malvern) was used to measure the zeta potential of the hybrid micelle solutions at room temperature. The concentration of copolymer mixtures after dialysis was adjusted to 0.2 mg/mL for the measurements.

2.6. Determination of drug content in the micelles

To quantify the amount of drug encapsulated, the micelles were broken up by adding 2 mL DOX-loaded micelle solutions into 8 mL DMF. The obtained solution was analyzed by an ultraviolet (UV) spectrometer (UV-2102PCS, Unico). The characteristic absorbance of DOX at 485 nm was recorded and compared with a standard curve generated from a DMF/H_2O (v/v = 4/1) mixture with DOX concentrations varying from 0 to 100 μg/mL.

2.7. In vitro drug release studies

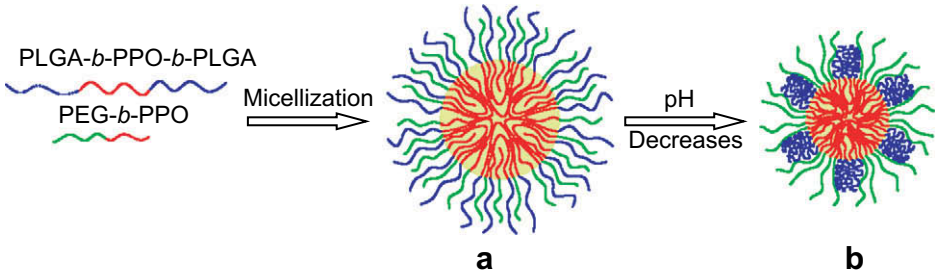
A fixed amount of DOX-loaded micelle solution suspended in dialysis bags was placed into buffer solution with required pH value. Then the samples were laid in a shaking bath at 70 rpm, at 37 °C and 20 °C, respectively. The buffer solution was replaced by fresh one periodically and measured to obtain the amount of released DOX. UV absorbance was measured at 485 nm and the DOX concentration was determined according to the standard curves of DOX solution at different pH values.

3. Results and discussion

This paper consists three sections: in the first section, PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO were used to prepare hybrid micelles. Once micelles prepared, we characterized the structure and also studied their stimuli-responsive properties. In the second section, we simulated the aggregation behavior using molecular dynamics (MD) to verify the experimental observations. In the last section, we investigated drug loading and releasing profiles of the micelles as functions of micelle structures and external stimuli.

*3.1. Micellization of PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO mixtures*

Since the PPO blocks of the PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO are hydrophobic in aqueous solution at room temperature, self-



Scheme 1. Schematic representation of the models proposed for the PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO hybrid micelles formed under different conditions. (a) basic and neutral condition, (b) acidic condition.

assembly occurs in water, leading to micelles in water with PPO as cores and PLGA and PEG as mixed coronas. The micellar structure was characterized by ^{1}H NMR, zeta potential measurement, and DLS.

^{1}H NMR was used to characterize chemical structure of solvated coronas in micelles. As shown in Fig. 1, the spectra for PLGA-*b*-PPO-*b*-PLGA unimers in $CDCl_3$ (Fig. 1a), micelles in D_2O (Fig. 1b) and hybrid micelles of PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO in D_2O (Fig. 1c) are compared. By the comparison of Fig. 1a and b, one can see, once PLGA-*b*-PPO-*b*-PLGA micelles formed (see Fig. 1b), the intensity of the signals due to PPO blocks (1.1, 3.5, 3.6 ppm) relative to those due to PLGA (2.1, 2.5 ppm) is reduced remarkably, indicating that the PPO blocks aggregated into the cores of the micelles and their chain mobility is restricted compared to the PLGA coronas. For the hybrid micelles in D_2O (see Fig. 1c), a signal due to PEG (3.7 ppm) appears in addition to those attributed to PLGA, indicating micelle shells consist a mixed polymer chains of PEG and PLGA. It is important to note that the signal of α-proton in PLGA block clearly appeared at 4.2 ppm (see Fig. 1c), which indicates that PLGA blocks take a coil chain conformation and remain solvated in aqueous media [50].

Zeta potential measurements were used to further confirm the corona structures of the micelles. We performed four experiments on the micelles with different ratios of the two copolymers. The results are illustrated in Fig. 2. As shown in the figure, PLGA-*b*-PPO-*b*-PLGA micelles possess negative zeta potential due to COO^- groups in the PLGA corona chains, while neutral zeta potential was observed for PEG-*b*-PPO micelles as expected. For the micelles with mixed copolymers, their zeta potentials are always negative and the potential is proportional to the amount of PLGA in the mixtures (see Fig. 2), which support that PLGA and PEG are mixed as corona chains.

One interesting feature of these hybrid micelles is that PLGA chains can undergo a transformation between coil conformation and α-helix depending on the pH of the solution [51–53]. We have proved this transformation using Circular Dichroism (CD) experiments on PLGA-*b*-PPO-*b*-PLGA micelle solutions (see Supplementary information). The CD spectrum at pH = 11.4 shows a typical curve, characteristic of a random coil conformation. The random coil PLGA is flexible, hydrophilic and in an extended form due to the electric repulsion between the LGA residues. When the pH value is decreased, the intensity of the coil characteristic peak decreases obviously at pH = 7.0, indicating that the coil to helix transition of PLGA blocks starts around pH = 7.0. Further decreasing pH, PLGA chains transform to helix form and tend to take a shrunk form with lower hydrophilicity [54–56] (for details of the CD data, see Supplementary information).

We also examined structure variation of the hybrid micelles as a function of pH using DLS. The results are summarized in Table 1. Because PEG chains are shorter than PLGA in the hybrid micelles, one can imagine that the conformation change of PLGA can effectively influence the size of the aggregates. As shown in the table, we indeed found that the hydrodynamic radii of the micelles dropped obviously in acidic conditions due to the transformation of PLGA from extended coil chains to shrunk α-helix. It is worth to mention that the aggregates are still in colloidal state despite the collapse of PLGA because PEG remains soluble at lower pH.

Another interesting point of the micelles is that the degrees of dehydration of PPO cores are sensitive to temperature. Higher temperature makes PPO cores more hydrophobic, lower temperature can swell the cores [57–59]. Therefore the hybrid micelles are also responsive to temperature that was proved by DLS (see Table 1). As shown in the table, we can see the micelles enlarged when temperature dropped to 20 °C as a result of temperature-induced swelling of PPO cores.

3.2. Molecular dynamics (MD) simulations of co-micellization

To verify the experimental results, we simulated the aggregation process using molecular dynamics (MD). In order to capture the essential features of the PLGA-*b*-PPO-*b*-PLGA triblock copolymer molecule and PPO-*b*-PEG diblock copolymer molecule, an $A_{10}B_7A_{10}$ triblock copolymer and a B_1C_3 diblock copolymer are constructed in MD simulations, respectively. The details of computation work are described in Supplementary information. At higher pH values, due to the repulsion among the LGA residues, a repulsive cut-off for the A–A interaction was considered in the simulations. Fig. 3a shows the simulated structure of the hybrid micelles. As shown in the figure, the B-blocks of the $A_{10}B_7A_{10}$ and B_1C_3 copolymers form the cores of the micelles and the cores are homogeneously surrounded by A- and C-block as corona. When the pH value is decreased, the polypeptide chains tend to be collapsed and become insoluble. In this sense, attractive interaction between A-blocks becomes dominant and an attractive cut-off for the A–A interaction is adopted. This results in a phase separation between A- and C-block. As can be seen in a snapshot in Fig. 3b, the A- and C-block segregate within the corona. Meanwhile, the A-blocks are shrunk.

Table 2
Drug-loading contents and particle size of DOX-loaded PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO hybrid micelles

PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO(weight ratio)	Hydrophobic chain [mol%]	Loading content[a] [wt%]	Loading efficiency[b] [%]	R_h of drug-loaded micelles [nm]	R_h of blank micelles [nm]
10/0	25.8	18.0	43.9	92.1	42.2
8/2	24.7	17.0	41.2	95.4	47.7
6/4	24.5	19.3	44.2	85.0	48.8
5/5	24.5	16.6	39.3	89.2	50.1
4/6	24.4	18.3	44.8	90.0	61.7
2/8	24.4	18.7	44.1	92.2	69.5
1/9	24.4	16.6	49.8	92.1	73.4

[a] The loading content was defined as the ratio of the weight of the loading drug to the weight of drug and copolymers in drug-loaded micelle solution.
[b] The loading efficiency was defined as the ratio of the weight of loaded drug to the initially added drug.

Summarizing both experimental findings and simulation results, we proposed a model of the hybrid micelle structures formed by PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO mixtures. In water, the hydrophobic PPO blocks from PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO aggregated into the micellar cores. The PLGA and PEG blocks associated leading to coronas with mixed polymer chains, as evidenced by ^{1}H NMR spectra, zeta potential measurements, as well as MD simulations.

In neutral and basic solutions, the PLGA blocks took random coil conformation as revealed by CD spectra, which are fully solvated and randomly mixed with the hydrophilic PEG blocks (see Scheme 1a). At lower pH, the random coil conformation of PLGA blocks transformed to α-helix and their solubility dropped. Consequently, microphase separation between the PLGA and PEG blocks occurred within the corona of the micelle, and the aggregation of PEG chains formed channels connecting the micelle core and outer milieu (see Scheme 1b). In addition, the PPO cores can respond to temperature. At a relative higher temperature, PPO became dehydrated leading to compact cores[60–62] and smaller aggregates as revealed by DLS measurements.

The pH stimulated PLGA conformation change and temperature-dependent hydration of PPO can tune both corona and core structures, but will keep integration and colloidal stability of the micelles. These properties may be useful for the design of drug carriers [18]. It is especially interesting to know whether the mixed coronas involving PLGA can be used to manipulate drug releasing rate within a narrow pH range from 7.4 to 5.0.

3.3. Drug loading and releasing studies

For this study, doxorubicin hydrochloride (DOX-HCl) was used which is an anti-cancer drug. As shown in Table 2, the loading

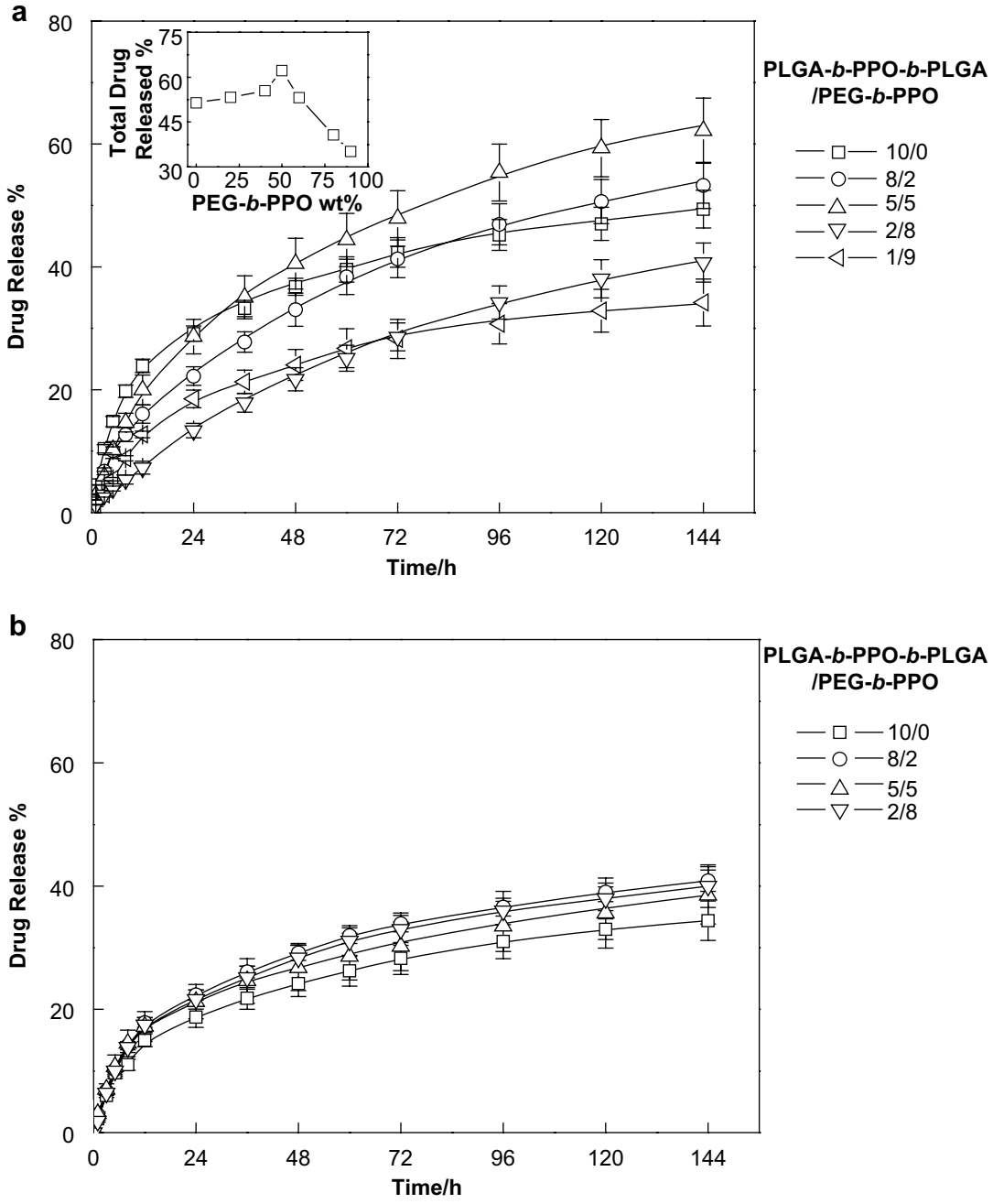


Fig. 4. Release of DOX from hybrid micelles formed by mixtures of PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO with various compositions at (a) pH = 5.5, 37 °C and (b) pH = 7.4, 37 °C.

efficiency and the amount of drug-loaded in the hybrid micelles are irrelevant to the weight ratio of PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO. These results can be attributed to a similar amount of hydrophobic PPO segments in the hybrid micelles regardless of the ratios of two polymers, because the hydrophobic interactions between core blocks and hydrophobic molecules are responsible for the drug loading [32,33,63]. The hydrodynamic radius (R_h) of drug-loaded hybrid micelles characterized by DLS are ca. 90 nm that is larger than blank micelles, suggesting that drugs have been entrapped.

The drug releasing profiles were then studied at 37 °C in a solution with pH of 5.5. Fig. 4 shows the effect of the composition of the hybrid micelles on the releasing kinetics of DOX. As shown in Fig. 4a, the micelles with higher PEG-*b*-PPO content have a slower releasing rate (the curve with PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratio of 1:9). As the PLGA-*b*-PPO-*b*-PLGA content increased, the release rates accelerated (the curve with PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratio of 2:8). A maximum rate is reached when PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratio is 5:5 (see Fig. 4a). Further increasing the content of PLGA-*b*-PPO-*b*-PLGA starts to decrease the releasing rate (the curve with PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratio of 8:2 and 10:0). This composition dependence of the releasing rate can be viewed more explicitly in the inset plot of Fig. 4a. As can be seen, with increasing the content of PEG-*b*-PPO, the total releasing amount increased and reached a maximum value for the aggregates containing 50% of PEG-*b*-PPO, then deceased gradually.

A set of similar experiments were performed at pH of 7.4. The results are shown in Fig. 4b. As shown in the figure, under this condition, we found that the releasing rate was almost irrelevant to the composition of two polymers in the micelles. From these two set of experiments under pH of either 5.5 or 7.4, we learned that hybrid micelle structure can facilitate drug releasing only under acidic conditions. In other words, the corona containing channels formed by PEG chain aggregation can help drug diffusion from the core of micelles.

The influence of pH on the release rate was further investigated by performing two set of experiments on the micelles with PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratios of 5:5 and 1:9. Drug releasing profiles under four pH conditions were compared in Fig. 5a, b. As shown in Fig. 5a, for the micelles with equal amount of two copolymers, the releasing rates are almost the same at pH of 7.4 and 8.4. However, under acidic situation, it becomes obvious that lower pH accelerates the releasing rate dramatically. For the micelles with small amount of PLGA-*b*-PPO-*b*-PLGA, pH in a wide range from basic to acidic did not change releasing rate at all as shown in Fig. 5b. This further supports that the micro-structure of the corona in the hybrid micelles stimulated by pH is an important factor determining drug releasing profiles.

The influence of temperature on the releasing rate was also studied, because the shrinkage of PPO cores at higher temperatures could also help a quick release of DOX [61,62]. We studied this effect on hybrid micelles (PLGA-*b*-PPO-*b*-PLGA:PEG-*b*-PPO ratio of 5:5) at two temperatures of 20 °C and 37 °C, respectively. As shown in Fig. 6, one can see that the influence of temperature on the releasing rate is minor at pH of 7.4, but becomes significant at pH of 5.5 (see Fig. 6). This is because that microphase separation, which can result in a PEG channel formation in corona, takes place at lower pH, a pass way for the rapid drug release forms. This result again suggested that the corona structure changes of the

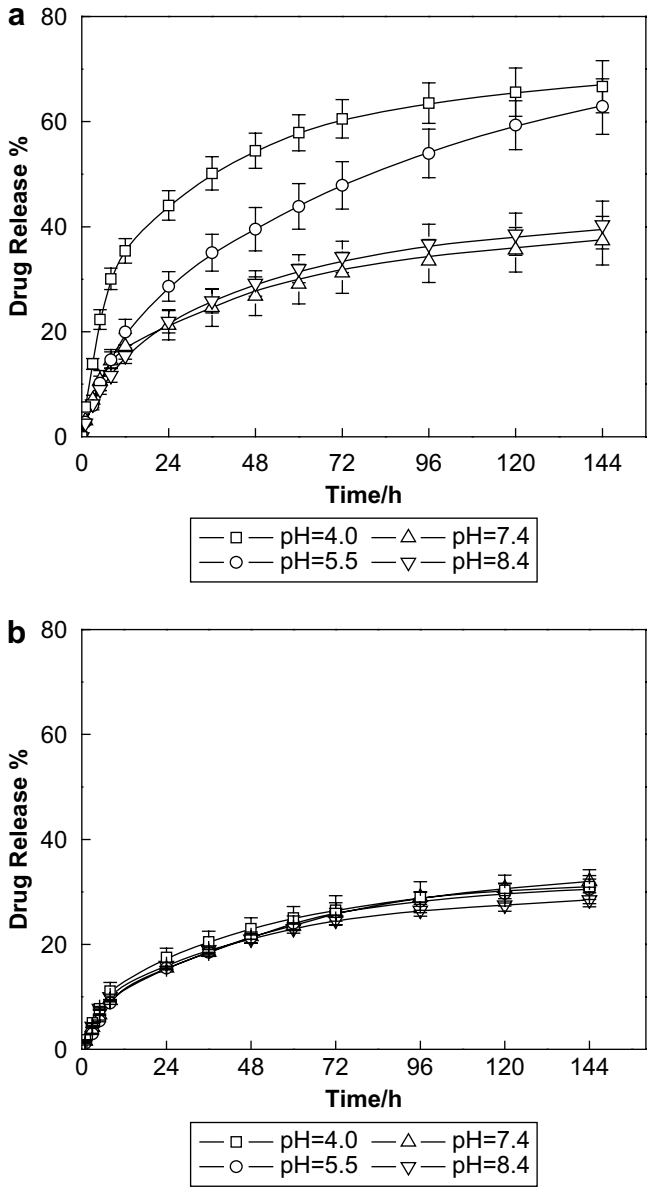


Fig. 5. Release of DOX from hybrid micelles formed by mixtures of PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO with ratios of (a) 5/5 and (b) 1/9.

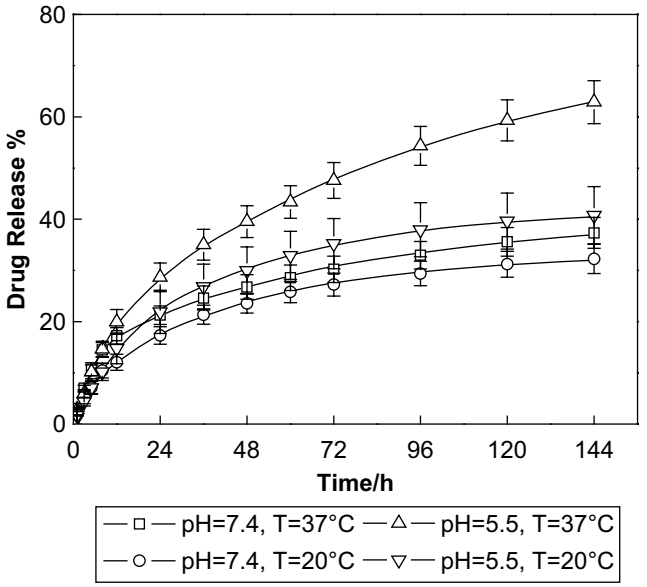
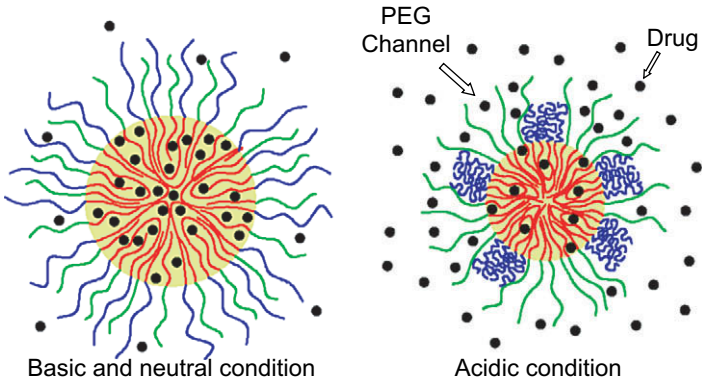


Fig. 6. Release of DOX from hybrid micelles formed by mixtures of PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO with ratio of 5/5 at various pH and temperatures.



Scheme 2. Schematic representation of the release mechanism proposed for the PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO drug-loaded hybrid micelles.

hybrid micelle played an important role in manipulating drug releasing rates.

Based on the results, we proposed a possible mechanism (see Scheme 2) to rationalize our discovery. First, as previous reported [64,65], the conformation and solubility change of the PLGA corona chains at lower pH can cause a stress on the core of the micelles, which gave rise to a core distortion inducing a leakage of loaded drugs and rapid drug release [65]. Second, as an interesting discovery of this paper, we found the ratio of PLGA and PEG chains in the corona played an important role to accelerate releasing rate presumably due to a channel effect. The PEG domain with higher solubility connected the micelle core and outer milieu, serving as a pass way for a rapid diffuse of DOX molecules. The diffusion of hydrophobic drug DOX through hydrophilic PEG chains is possible as described in many previous literature [66–68], while the collapsed PLGA chains under acidic conditions could retard the release of DOX [69–72]. As a result, the channel of PEG was formed for accelerated drug release. As shown in Scheme 2, at higher pH, PLGA adopted a coil conformation, there is no stress imposed on the cores of the micelles and no PEG channels formed. Therefore, the composition variation of the two polymers barely influenced releasing rate (see Fig. 4b). At acidic conditions, the conformation change of PLGA generated stress on the core of the micelles for a faster releasing (for the hybrid micelles, the drugs are squeezed throughout the PEG channels under the pressure imposed by the collapsed PLGA chains). Higher content of PLGA chains can generate higher stress force, but results in smaller domain of PEG channels. These two structure parameters have to be compromised against each other for a faster releasing rate. Thus, a maximum rate was observed for the sample with an appropriate amount of PEG-*b*-PPO (see Fig. 4a). The observed channel effect is also clearly evidenced by the experiments as a function of temperature (Fig. 6). At a higher pH value, PLGA and PEG chains mixed randomly and there are no PEG channels. As a result, we found that the influence of temperature on the releasing rate is small. Once dropping pH, we observed a marked temperature-dependent releasing rate, which can be attributed to the formation of PEG channels.

The observed channel effect induced by the phase separation of micellar coronas is interesting, because the channels can be constructed and destructed within a narrow pH range without loosing colloidal stability. In practice, most pathological processes exhibit a decrease in pH from 7.4 to 5.0 [20]. Contrary to the normal blood pH of 7.4, the extracellular pH values in tumorous tissues are determined to be around 6.5–7.0. Drug nanocarriers can enter living cells via endocytosis, and the intracellular environment is typically acidic (pH 5.0–6.0). This pH change in pathological processes can switch on the formation of channels in the corona of hybrid micelles due to the PLGA chain conformation transition and solubility change, accelerating the drug releasing rate. We therefore anticipate that the hybrid micelles will be a promising candidate as drug carrier with more sophisticated and smarter controlled release behavior in applications of drug delivery systems.

4. Conclusions

We prepared hybrid micelles through the self-assembly of PLGA-*b*-PPO-*b*-PLGA and PEG-*b*-PPO. The resulting micelles contain hydrophobic PPO cores and mixed coronas of PLGA and PEG. This structure was characterized by using ^{1}H NMR, zeta potential measurements, DLS and verified by using MD simulations. Further studies of this type of micelles as drug carriers led to the discovery that the mixed corona chains containing PLGA and PEG play an important role in tuning drug releasing rate. For the micellar drug carriers with an appropriate PLGA-*b*-PPO-*b*-PLGA/PEG-*b*-PPO composition, the drug releasing rates were significantly accelerated when pH of the solution changed from 7.4 to 4.0. We attribute this discovery to the conformation change of PLGA at lower pH, which caused the phase separation of PLGA and PEG leading to the formation of the PEG channels in the micelle coronas. These channels facilitated the drug diffusion. In addition to this discovery, the reported hybrid micelles are particularly interesting for clinical applications for two reasons: (1) all blocks generally have good biocompatibility, (2) the pH range (from 7.4 to 4.0) used to tune drug releasing rate just fits that in pathological processes. Therefore we can design a drug carrier which can keep the integration of the aggregates and retain the drug in blood stream, but quickly release it at disease sites.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biomaterials.2008.09.010.

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