Research Article

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Morphological transition of amphiphilic block copolymer/PEGylated phospholipid complexes induced by the dynamic subtle balance interactions in the self-assembled aggregates

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Abstract: Recently, there has been an increasing interest in the control of morphological transition of block copolymer aggregates. Here, we report how to control the morphological transition of methoxy polyethylene glycol-poly(D,L-lactic acid) (PDLLA-MPEG) by adding 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)] (DSPE-PEG). In the aggregates formed by dialyzing DSPE-PEG/PDLLA-MPEG mixed solutions against water, the two hydrophobic fatty acid tails of DSPE-PEG will preferentially anchor to the hydrophobic segment end of PDLLA-MPEG by interdigitating between these two tails and PDLLA segments. Consequently, DSPE-PEG and PDLLA-MPEG will form “ABA” temporary supra-amphiphiles in which A represents a poly(ethylene glycol) (PEG) chain segment and B is a mixed hydrophobic segment composed of PDLLA and DSPE segments; the repulsive force derived from the PEG segments of DSPE-PEG can affect the stability of “ABA” temporary supra-amphiphiles. Our results show that the dynamic subtle balance between the number of “ABA” temporary supra-amphiphiles formed and the strength of repulsive force between the PEG segments of DSPE-PEG drives the morphological structure of DSPE-PEG/PDLLA-MPEG aggregates to change from micelles to vesicles, then to semi-vesicles and finally to mixed micelles, with increasing DSPE-PEG additions.

Keywords: amphiphilic block copolymer, self-assembly, morphological transition, interpolymers complexation, dynamic subtle balance interaction

1 Introduction

Self-assembly of amphiphilic block copolymers into nanostructures in selected solutions has been extensively studied and shows promising performance in drug delivery applications (1,2). A variety of self-assembled structures, such as core-shell spherical micelle, vesicle, rod-like and lamellar or other supramolecular structures, of amphiphilic block copolymers can be prepared in aqueous solution, by tuning a subtle balance between the hydrophobic segments and hydrophilic segments and designing suitable copolymer backbones (3–8). Recently, there has been a growing interest in the control of morphological transition between these self-assembled structures of amphiphilic block copolymers due to their great potential in controlled drug-delivery systems (9–11). Although self-assembly behavior of amphiphilic block copolymers in aqueous solution has been well understood and the effective rules have been developed to guide scientists to synthesize suitable copolymers to obtain the desired self-assembled structures (12–15), it is still a challenge to control the morphological transition of self-assembly structures effectively and conveniently. Recently, some studies on the development of nanoparticle/amphiphilic block copolymer nanocomposites indicate that the incorporation of nanoparticles in a polymeric matrix may be used to manipulate the morphological transition of amphiphilic block copolymer aggregates by varying the relative volume ratio of hydrophobic block/hydrophilic block (16–18). Co-assembly of nanoparticle and amphiphilic block copolymer can give rise to different functional nanostructures with extensive applications in biomedical fields. However, the morphological transformation behavior of these nanostructures is usually unpredicted theoretically.

An alternative effective way to direct the morphological transition of amphiphilic block copolymer aggregates is mixing two amphiphilic block copolymers or a block copolymer with a homopolymer in which the intrachain cross-linking of the hydrophilic segments of two polymers can occur through the noncovalent

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interactions (19–22). Such interpolymer complexation makes it convenient to manipulate the morphology of the obtained polymer aggregates by changing the hydrophilic/hydrophobic balance of amphiphilic block copolymers. The copolymer mixtures can be self-assembled into more advanced structures than that of the corresponding single block amphiphilic copolymer system because the interpolymer complex via noncovalent interactions can form a totally different chain structure from that of the original polymer. The as-formed chain structure constitutes new “temporary amphiphiles” and has a different hydrophilic/hydrophobic balance value from that of the original amphiphilic copolymer, which results in the morphological transitions of self-assembled structures. Such strategy of constructing temporary amphiphiles avoids the tedious chemical synthesis process and is a more environmentally friendly approach.

The interpolymer complexation of copolymer mixtures that can adjust the morphological transition reported so far is driven mainly by the electrostatic interaction or hydrogen bonding within the hydrophilic segments of polymers. These relatively strong and directional noncovalent interactions usually require a careful design of polymer with appropriate interaction segments between the polymer mixtures so that they can self-assemble into various morphologies and structures. It should also be pointed out that the hydrophilic segments of amphiphilic block copolymers will inevitably be changed by this strategy. However, these hydrophilic segments of amphiphilic block copolymers usually play a key role in the drug delivery system. For instance, hydrophilic poly(ethylene glycol) (PEG) segments have been commonly used to make amphiphilic block copolymer aggregates with a longer circulation half-life and less nonspecific accumulation in a healthy tissue (23,24). Recently, it is interesting to find that tuning the hydrophobicity or the flexible and rigid hydrophobic moieties of amphiphilic block copolymers can also provide a way for the control of self-assembly behavior of amphiphiles (25,26). However, the modification of hydrophobic segments as an effective means to adjust the morphological structure changes of amphiphilic block copolymers is very limited.

Phospholipids are the most typical kind of natural amphiphiles in cell membranes that consist of one hydrophilic head and two hydrophobic fatty acid tails. This unique geometric molecular structure makes phospholipids as basic building blocks to form cell membrane bilayer structures and act as binding sites for other biological molecules (27,28). Phospholipids have

also been widely used to prepare hybrid stealth micelles or vesicles with synthetic amphiphilic block copolymers to tailor the structure asymmetry or biocompatibility of these supramolecular nanostructures for pharmaceutical and biomedical applications (29–32). Generally, the two-hydrophobic tailed phospholipids prefer forming bilayer structures by interdigitating between them. Forming a bilayer structure is usually a key step for amphiphilic copolymers to transform from micelles to vesicles. However, this two-hydrophobic tailed structure leads to structural incompatibility between phospholipids and linear copolymers. As a result, the structural incompatibility in combination with the incompatibility in the hydrophilic layer between the natural phospholipids and the synthetic amphiphilic copolymers usually leads the phase separation to occur and presents the lipid-rich domain with the bilayer structure in the formed phospholipid/synthetic amphiphilic copolymer hybrid aggregates. But no obvious controllable morphological transition has been observed in the hybrid aggregates during the addition of phospholipid to the synthetic amphiphilic copolymers.

PEGylated phospholipids that are prepared by grafting PEG to the head groups of phospholipids provide a way to tune the head group hydrophilicity/hydrophobicity of phospholipids and can improve their solubility in water and decrease the interaction between phospholipids themselves. As such, PEGylated phospholipids have found to be promising in steric stabilization applications of nanoparticles or carbon nanotubes because their two hydrophobic tails can anchor to the surface of nanoparticles or carbon nanotube while the long hydrophilic PEG block can form a sterically repulsive corona around their surfaces (33,34). It is interesting to explore how the two hydrophobic tails of PEGylated phospholipids can interact with linear amphiphilic copolymers. If the interdigitating hydrophobic interaction ability of the two hydrophobic tails of PEGylated phospholipids can be retained and made to interact with the hydrophobic segments of amphiphilic copolymers to form a bilayer structure, PEGylated phospholipids may possibly be used to regulate the controllable morphological transition of amphiphilic copolymer aggregates. In this study, the most typical amphiphilic block copolymers, methoxy polyethylene glycol–poly(D,L-lactic acid) (PDLLA–MPEG) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethylene glycol)] (DSPE–PEG), are used to explore the possibility of DSPE–PEG to mediate the morphological transition of self-assembled structures from their mixtures. The effect of DSPE–PEG on the morphology

and size of self-assembled structures was studied by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Upon increasing the ratio of DSPE-PEG/PDLLA-MPEG, it is found that the morphology of self-assembled structures from the DSPE-PEG/PDLLA-MPEG mixtures changes from spherical micelles into vesicles, semi-vesicles and then into mixed micelles. Based on the obtained data, a possible explanation for the method by which DSPE-PEG mediates the morphological transition of DSPE-PEG/PDLLA-MPEG complex aggregates was developed.

2 Materials and methods

2.1 Materials

1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy-(polyethylene glycol)] (DSPE-PEG2k, DSPE-PEG1k, DSPE-PEG5k) was purchased from Shenzhen Xingjiafeng Technology Co. Ltd (China). Monomethoxy poly(ethylene glycol)-*block*-poly(D,L-lactide) (PDLLA10k-MPEG2k) was obtained from Jinan Dai-gang Biological Technology Company (Jinan, China). Doxorubicin hydrochloride (DOX-HCl) was obtained from Beijing Hua-feng United Technology Company (Beijing, China). Triethylamine (TEA) and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Dialysis tubes (molecular weight cutoff = 8,000–12,000 Da) were purchased from Shanghai Yuanye Biological Technology Company (Shanghai, China). All solvents and reagents were of analytical grade and used as such without further purification.

2.2 Methods

2.2.1 Preparation of DSPE-PEG/PDLLA-MPEG complex aggregates

DSPE-PEG/PDLLA-MPEG complex aggregates were prepared by a membrane dialysis method. Briefly, 10.0 mg of DOX-HCl was dissolved in 5 mL of DMSO and then 0.5 mL of TEA was added into the solution. The mixed solution was stirred overnight to remove the hydrochloride in DOX-HCl. Subsequently, 40.0 mg of MPEG-PDLLA and different amounts of DSPE-PEG were added into the solution (the mass ratio of DSPE-PEG to PDLLA-MPEG was changed from 0:1 to

0.02:1, gradually). Next, the mixtures were stirred under magnetic stirring at room temperature to form a uniform solution. Then, the mixed solutions were transferred to a dialysis tube (molecular weight cutoff = 8,000–12,000 Da) and dialyzed against deionized water for 72 h to remove the organic solvent. Finally, the different mass ratios of DSPE-PEG/PDLLA-MPEG complex aggregates were obtained.

2.2.2 Characterization of DSPE-PEG/PDLLA-MPEG complex aggregates

TEM measurements were performed with a JEOL JEM-2010 transmission electron microscope operating at an accelerating voltage of 100 kV. The TEM samples were prepared by depositing one drop of complex aggregate solutions onto a 200-mesh copper grid which was carbon-coated and then dried in air overnight at room temperature.

The hydrodynamic diameters of DSPE-PEG/PDLLA-MPEG complex aggregates were measured using Zeta sizer (Nano ZS90 instrument; Malvern Instruments, UK). The results were collected using the intensity distribution at a fixed scattering angle of 90° at 25°C.

3 Results and discussion

3.1 Characterization of DSPE-PEG/PDLLA-MPEG complex aggregates formed at different DSPE-PEG additions

The effect of DSPE-PEG on the morphology and size of DSPE-PEG/PDLLA-MPEG complex aggregates was studied by TEM. Figure 1 shows the representative TEM images of DSPE-PEG2k/PDLLA-MPEG complex aggregates at different mass ratios of DSPE-PEG2k/PDLLA-MPEG. As expected, the morphological structure of DSPE-PEG2k/PDLLA-MPEG complex aggregates could be changed by increasing the amount of DSPE-PEG addition. For the pure MPEG-PDLLA molecules, a structure of spherical micelles with good dispersity was formed and the calculated average size of micelles was about 231 nm, as shown in Figure 1a. This can be ascribed to their amphiphilic property which makes them spontaneously self-assemble into micelles in the aqueous solution. When the mass ratio of DSPE-PEG2k to PDLLA-MPEG was 0.0005:1, the DSPE-PEG2k/PDLLA-MPEG complex aggregates showed irregular spherical micelles (Figure 1b) with an

Figure 1: TEM images of aggregates formed from DSPE-PEG2k/MPEG-PDLLA mixtures with different mass ratios of DSPE-PEG2k/PDLLA-MPEG: (a) 0:1, (b) 0.0005:1, (c) 0.001:1, (d) 0.005:1, (e) 0.01:1 and (f) 0.02:1.

average size of 283 nm. When the mass ratio of DSPE-PEG2k to PDLLA-MPEG was increased to 0.001:1, the morphology of complex aggregates had a significant transition, where a structure of vesicles with diameters ranging from 2 to 4 μm was obtained, as shown in Figure 1c. As the mass ratio of DSPE-PEG2k/PDLLA-MPEG reached 0.005:1, some destroyed vesicles were found in the TEM images, and it could be observed that some integral vesicles coexisted with the partially ruptured ones, as indicated in Figure 1d. This phenomenon indicated that the stability of vesicles at this mass ratio becomes poor. Then, with the further increase in mass ratio of DSPE-PEG2k/PDLLA-MPEG to 0.01:1, almost no intact vesicles were found, and the morphology of complex aggregates showed semi-vesicle structure (Figure 1e). The semi-vesicular morphological structure has already been mentioned in the literature as a transition state in the structural transition between vesicles and micelles of polymers (35). However, it is interesting to point out that it is the first time to find the existence of this morphological structure experimentally. At an even higher mass ratio of DSPE-PEG2k/PDLLA-MPEG (0.02:1), it was surprised to find that a structure of irregular spherical micelles, similar to that of the mass ratio at 0.0005:1, occurred again. Although the morphology of DSPE-PEG2k/PDLLA-MPEG complex aggregates obtained under these two ratios is similar, the difference in composition ratios of these two complex aggregates is remarkably large and in turn the respective micelle properties may be completely different.

It is evident that the addition of DSPE-PEG2k leads to the morphological transition of PDLLA-MPEG aggregates.

To further verify the effect of DSPE-PEG2k additions on the morphology of complex aggregates, the DLS test was used to determine the size distribution of DSPE-PEG2k/PDLLA-MPEG complex aggregates as indicated in Figure 2. The DLS results showed that PDLLA-MPEG micelles have an average size of 280 nm at 25°C. When DSPE-PEG2k was added in the 0.0005:1 mass ratio, the average size of DSPE-PEG2k/PDLLA-MPEG complex aggregates increased to 351 nm. Then, with the further increase in DSPE-PEG2k content, the DSPE-PEG2k/

Figure 2: Size distributions of DSPE-PEG2k/PDLLA-MPEG complex aggregates prepared from the DSPE-PEG2k/PDLLA-MPEG mixed solutions at different mass ratios of DSPE-PEG2k/PDLLA-MPEG.

PDLLA-MPEG complex aggregates exhibited a dual size distribution, with a dominant size in the nanometer range and the other one in the micrometer range. Consistent with the TEM results in Figure 1, the size distribution changes in DLS data are attributed to the evolution of the complex aggregates from micelles to vesicles and then semi-vesicles. At a higher mass ratio of 0.02:1, the DLS data of DSPE-PEG2k/PDLLA-MPEG complex aggregates just exhibit a single distribution with an average size of 296 nm. This result means that the complex aggregates completely changed into micelle structure again, as indicated in Figure 1f. Consequently, TEM images and DLS data both showed that the addition of DSPE-PEG2k is an effective way to regulate the morphological transition of PDLLA-MPEG self-assembled structures.

The aforementioned results obviously indicated that the gradual increase in DSPE-PEG2k additions resulted in the morphological transition of DSPE-PEG2k/PDLLA-MPEG complex aggregates from micelles to vesicles, semi-vesicles and then to mixed micelles again. To investigate how the DSPE-PEG2k molecules affect the self-assembly structure of DSPE-PEG2k/PDLLA-MPEG mixtures and further explore the interaction between DSPE-PEG2k and PDLLA-MPEG polymers in solutions, the dynamic mixing process of DOX-loaded PDLLA-MPEG micelle solutions with DSPE-PEG2k molecules was monitored by TEM. In this process,

MPEG-PDLLA/DOX mixed solution was first dialyzed against aqueous solution to form micelles and then DSPE-PEG2k was added to the PDLLA-MPEG micelle solutions and mixed for different time periods. Figures 3 and 4 show the TEM images of DSPE-PEG2k and PDLLA-MPEG micelles mixed at different time periods with the mass ratio of 0.001:1 and 0.02:1, respectively. As shown in Figures 3a and 4a, only evenly dispersed spherical micelles could be observed at 0 h, i.e., no DSPE-PEG2k was added into the amphiphilic block polymer micelles. With the increase in mixing time to 1 h, the aggregates were still spherical micelles but there was adhesion between the spherical micelles as shown in Figure 3b (the mass ratio of DSPE-PEG2k/PDLLA-MPEG was 0.001:1); a similar phenomenon was also observed at this mixing time in the case where the mass ratio of DSPE-PEG2k/MPEG-PDLLA was 0.02:1 (Figure 4b). Both the vesicles began to be observed in TEM images after the addition of DSPE-PEG2k to PDLLA-MPEG micelle solutions for 6 h at these two different mass ratios (Figures 3c and 4c). However, as the mixing time increased further, the morphological changes of aggregates formed from the DSPE-PEG2k molecules and PDLLA-MPEG micelle mixed solutions showed different evolution at different mixed mass ratios. When the mass ratio of DSPE-PEG2k/PDLLA-MPEG was 0.001:1, the number of vesicles observed in TEM images increased with

Figure 3: TEM images of aggregates formed from DSPE-PEG2k and PDLLA-MPEG micelle (at a mass ratio of 0.001:1) mixed solutions at different mixing time: (a) 0 h, (b) 1 h, (c) 6 h, (d) 12 h and (e) 24 h.

the further increase in mixed time and finally showed a similar TEM image to that of Figure 1c. In contrast, in the case where the mass ratio of DSPE-PEG2k/PDLLA-MPEG was 0.02:1, the number of vesicles observed in TEM images did not only increase with increasing mixed time but also the vesicles observed in TEM images gradually ruptured and disappeared, and eventually changed into micelles again. The time-dependent morphological evolution of aggregates formed by mixing PDLLA-MPEG micelle solutions and DSPE-PEG2k molecules demonstrated that the DSPE-PEG2k can gradually penetrate into the PDLLA-MPEG micelles which leads micelles to re-assemble into different new aggregates according to the different amounts of DSPE-PEG penetration. The hydrophobic DOX encapsulation in the micelles may enlarge the inner cores and cause more chains to be looped that may facilitate the DSPE-PEG2k to penetrate into the PDLLA-MPEG more easily (36). But during the mixing of PDLLA-MPEG micelles and DSPE-PEG2k, the morphological transition of aggregates formed from the PE-PEG and PDLLA-MPEG micelles mixed solutions, from micelles to vesicles or finally to mixed micelles, should be the result of the interaction between DSPE-PEG and PDLLA-MPEG, not the one due to

DOX release. It is because that the released DOX cannot shift the relative volume ratio of hydrophobic block/hydrophilic block to a high value, while the increasing hydrophobic block/hydrophilic block value is a key step for the transition of copolymers from micelles to vesicles. Therefore, these obtained results further confirmed that the addition of DSPE-PEG2k can effectively adjust the morphological transition of PDLLA-MPEG self-assembled structure and form various thermodynamically stable supramolecular structures.

3.2 Proposed possible mechanism of morphological transition of DSPE-PEG2k/PDLLA-MPEG complex aggregates

Interactions between two copolymers in a binary mixture of amphiphilic block copolymers mainly involve two ways. One is the occurrence of intrachain cross-linking of the hydrophilic segments of two copolymers through the noncovalent interactions, such as hydrogen

Figure 4: TEM images of aggregates formed from DSPE-PEG2k and PDLLA-MPEG micelle (at a mass ratio of 0.02:1) mixed solutions at different mixing time: (a) 0 h, (b) 1 h, (c) 6 h, (d) 12 h and (e) 24 h.

bonding or electrostatic interactions. In this case, the hydrophilic/hydrophobic balance of amphiphilic block copolymers changes, and this can be used for convenient regulation of the morphological transition of the obtained polymer aggregates (19–22). Other interaction between two amphiphilic block copolymers is that the hydrophobic segments of mixtures are packed together into hydrophobic aggregates by hydrophobic interactions. In the latter, co-micellization of two amphiphilic block copolymers is generally the most evident appearance. The structure of co-micellization of binary mixed amphiphilic block copolymers can be mixed micelles or demixed ones which can be easily and precisely regulated by the block length, block chemistry and the stoichiometry of the binary mixture (37,38). Moreover, mixed micelles formed from two amphiphilic block copolymers with obviously distinct hydrophobic lengths usually present ellipsoidal structures in which the inner cores are mainly occupied by the longer hydrophobic blocks while the shorter blocks straddle the core and corona interface by their hydrophobic block inserted in the inner core through the hydrophobic interaction (37). The difference between the two hydrophobic blocks also may cause the microphase separation of the inner core to occur, which induces the morphological change of mixed micelles. However, almost no obvious controllable morphological transition appears for such binary mixtures, mainly because of the relatively weak strength of the hydrophobic interaction and the poor selectivity between two hydrophobic blocks, which hinder the formation of a regular complex from a mixture of amphiphilic block copolymers.

If the assembled aggregates from a mixture of DSPE-PEG/PDLLA-MPEG also prefer to present mixed micelle structures with the DSPE-PEG concentrating in the core and corona interface as that usually observed in a binary mixture of amphiphilic block copolymers (37), the aggregates thus obtained will not be the ones with stable energy due to the structural incompatibility between two-hydrophobic tailed DSPE-PEG and linear PDLLA-MPEG. As it is well-known, due to their two-hydrophobic tailed structure, the phospholipids usually prefer to form bilayer structures by interdigitating between them. DSPE-PEG have a long hydrophilic PEG segment that improves their solubility in water and greatly decreases the hydrophobic interaction between DSPE-PEG themselves. But the interdigitating hydrophobic interaction ability of the two hydrophobic tails of DSPE-PEG should be retained when they interact with the linear PDLLA-MPEG. Consequently, the DSPE

segment of DSPE-PEG will be preferentially interdigitated with the end of linear hydrophobic PDLLA segment of PDLLA-MPEG through the hydrophobic interaction, when DSPE-PEG/PDLLA-MPEG mixtures are dialyzed against water. In this interaction way, the two fatty acid tails of DSPE-PEG can interact with three PDLLA segments of PDLLA-MPEG, so that it can obtain as much hydrophobic interaction as possible between the hydrophobic blocks of mixtures and decrease the structural inconsistencies of the hydrophobic aggregation region formed by the DSPE and PDLLA segments together. As such, interaction between the DSPE and PDLLA segments also facilitate the DSPE-PEG and PDLLA-MPEG to form “ABA” temporary supra-amphiphiles. This new building block may cooperatively assemble with PDLLA-MPEG into energetically stable bilayer supramolecular structures in which all the hydrophobic chains are fully contacted while the PEG segments of PDLLA-MPEG form the outer hydrophilic layer and the PEG segments of DSPE-PEG form the inner hydrophilic layer to provide steric repulsion to stabilize the hydrophobic aggregates. Consequently, the unique interaction between DSPE-PEG and PDLLA-MPEG makes them tend to form “ABA” temporary supra-amphiphiles and self-assemble into bilayer aggregates, which is a key step to transit from micelles to vesicles. Therefore, the addition of DSPE-PEG played an active role in the morphological evolution of DSPE-PEG2k/PDLLA-MPEG complex aggregates from micelles to vesicles. It is interesting to point out that the as-formed vesicle from DSPE-PEG2k/PDLLA-MPEG mixtures has an asymmetric structure in which the hydrophilic segments of DSPE-PEG are located in the inner chamber. However, it should be noted that the formation of hydrophilic layer from the PEG segments of DSPE-PEG is necessary to form bilayer structure aggregates, while the repulsive force derived from them in turn may affect the stability of “ABA” temporary supra-amphiphiles. Therefore, when only a small amount of DSPE-PEG is added to PDLLA-MPEG, the PEG segments of DSPE-PEG cannot form a hydrophilic layer with sufficient repulsive force to maintain the formation of the bilayer structured aggregates from the DSPE-PEG/PDLLA-MPEG mixtures. At this time, mixed micelles were formed from the DSPE-PEG/PDLLA-MPEG mixtures by inserting the two fatty acid tails of DSPE-PEG in the inner core. Presumably, the obvious difference in the hydrophobic block structures between DSPE-PEG and PDLLA-MPEG can lead the inner core of micelle to deform and present an irregular sphere structure as indicated in Figure 1b. When the addition

of DSPE-PEG is increased by a certain amount, the number of “ABA” temporary supra-amphiphiles formed is sufficient to form aggregates with a bilayer structure in which the PEG segments of PDLLA-MPEG form the outer hydrophilic layer and the PEG segments of DSPE-PEG form the inner hydrophilic layer to provide steric repulsion and stabilize the formed aggregates. In this case, the morphology of DSPE-PEG2k/PDLLA-MPEG complex aggregates presents vesicle structures as indicated in Figure 1c and d. On further increasing the amounts of DSPE-PEG addition, more “ABA” temporary supra-amphiphiles will form to facilitate the DSPE-PEG/PDLLA-MPEG mixtures assemble into vesicles, while the mutual repulsive force between the PEG segments of DSPE-PEG located in the inner chamber of vesicles also increases. It should be noted that the driving force for the formation of “ABA” temporary supra-amphiphiles from DSPE-PEG and PDLLA-MPEG mixtures is the weak van der Waals force. In addition, the interaction strength between the DSPE-PEG2k and PDLLA-MPEG is also weak due to the short hydrophobic tail of DSPE-PEG. Therefore, the increasing repulsive force in the inner chamber of vesicles with increasing DSPE-PEG2k additions would cause “ABA” temporary supra-amphiphiles to be unstable or even to be destroyed. The destruction of “ABA” temporary supra-amphiphiles will, in turn, lead the vesicles to be unstable or even disappear. Therefore, a continuous increase in the DSPE-PEG2k addition can further change the morphology of DSPE-PEG2k/PDLLA-MPEG complex aggregates. To avoid the conflict between the presence of stable “ABA” temporary supra-amphiphiles and the increasing repulsive force caused by the increasing DSPE-PEG2k in a closed chamber, DSPE-PEG2k/PDLLA-MPEG complex aggregates would change from vesicle structures to semi-vesicle structures as indicated in Figure 1e. The semi-vesicles that are nearly disks in structure can allow more “ABA” temporary supra-amphiphiles to exist in the aggregates because the repulsive force between the DSPE-PEG2k molecules in the as-formed structure is less than that in a vesicle structure. This decreased repulsive force between the PEG segments of DSPE-PEG in semi-vesicles is due to the fact that these PEG segments are not subject to the repulsive force from the opposite half of the sphere as that exists in vesicle structures. Semi-vesicles are present as a transition state in the structural transformation between vesicles and micelles of polymers and are important intermediate structures instead of bilayer membranes (35). Generally, semi-vesicles are in a thermodynamically unstable state, and these structures have not been observed in

experiment previously. Interestingly, the stable semi-vesicles can be formed at a suitable ratio of DSPE-PEG2k/PDLLA-MPEG since this structure can reconcile the conflicts between the number of stable “temporary shape amphiphiles” formed and the increasing repulsive force coming from the DSPE-PEG2k. However, if DSPE-PEG/PDLLA-MPEG mixtures still preferentially form “ABA” temporary supra-amphiphiles under a high ratio of DSPE-PEG2k/PDLLA-MPEG, then, in the subsequently forming supramolecular structure, the repulsive force generated by the PEG segment of DSPE-PEG will be large enough to cause the structure unstable. Under such conditions, the geometric molecular structure of DSPE-PEG does not play a key role in determining the hydrophobic interaction site between the DSPE-PEG and PDLLA-PEG. And the DSPE-PEG and PDLLA-PEG will be self-assembled into mixed micelles in which the hydrophilic segments of DSPE-PEG and PDLLA-PEG together form a hydrophilic layer while the hydrophobic tails of DSPE-PEG are inserted into the hydrophobic cores via the hydrophobic interaction. In such mixed micelles, although the hydrophobic interaction between DSPE-PEG and PDLLA-PEG is not the strongest, the effect of repulsive force resulting from the PEG segment of DSPE-PEG on their interaction is eliminated and thus allows the formation of a related more stable supramolecular structure. Thereby, DSPE-PEG2k/PDLLA-MPEG complex aggregates would further change from semi-vesicle structures to mixed micelle structures under a high ratio of DSPE-PEG2k/PDLLA-MPEG, as indicated in Figure 1f.

According to the above discussion, a schematic diagram of how the addition of DSPE-PEG regulates the morphological transition of PDLLA-MPEG self-assembled structures is outlined in Figure 5.

This model describes that the geometric molecular structure of DSPE-PEG makes DSPE-PEG and PDLLA-PEG preferentially form “ABA” temporary supra-amphiphiles; however, subsequently, the repulsive force between the PEG segments of DSPE-PEG resulting from the formation of a bilayer supramolecular structure by the “ABA” temporary supra-amphiphiles and PDLLA-PEG in turn affects the stability of the “ABA” temporary supra-amphiphiles. The dynamic subtle balance between the number of “ABA” temporary supra-amphiphiles formed and the repulsive force strength between the PEG segments of DSPE-PEG under different DSPE-PEG2k additions causes the morphological structure of DSPE-PEG2k/PDLLA-MPEG complex aggregates to transit from micelles to vesicles, semi-vesicles and then to mixed micelles with increasing

Figure 5: Schematic representation of the possible aggregate structures formed from DSPE-PEG2k/PDLLA-MPEG mixtures with increasing mass ratio: (a) pure micelles, (b) vesicles, (c) semi-vesicles and (d) mixed micelles.

ratios of DSPE-PEG2k/PDLLA-MPEG. The time-dependent morphological evolution of aggregates formed by directly mixing PDLLA-MPEG micelle solutions and

DSPE-PEG2k molecules at different ratios also provides evidence for the plausibility of this proposed model as outlined in Figure 5 (cf. Figures 3 and 4).

To further demonstrate the proposed model for the morphological transition of DSPE-PEG2k/PDLLA-MPEG complex aggregates, two other DSPE-PEG molecules with different PEG molecular weights (DSPE-PEG1k and DSPE-PEG5k) were used to investigate how the DSPE-PEG affect the self-assembly process of DSPE-PEG/PDLLA-MPEG mixtures. By only changing the molecular weight of PEG segments of DSPE-PEG, the hydrophobic interaction strength between the two fatty acid tails of DSPE-PEG and the three PDLLA segments of PDLLA-MPEG does not change, while in the bilayer supramolecular structures formed, the repulsive force generated by the PEG segment of DSPE-PEG become stronger or weaker based upon a longer PEG or a shorter PEG segment of DSPE-PEG. TEM images of DSPE-PEG/PDLLA-MPEG complex aggregates prepared from the different mass ratios of DSPE-PEG5k and DSPE-PEG1k to the PDLLA-MPEG are shown in Figures 6 and 7, respectively. The obtained TEM images showed that the changing trends toward morphology of DSPE-PEG/PDLLA-MPEG complex aggregates with increasing addition of DSPE-PEG5k (Figure 6) or DSPE-PEG1k (Figure 7) are both similar to that of DSPE-PEG2k effect on the morphology of DSPE-PEG2k/PDLLA-MPEG complex aggregates (Figure 1). However, it should be noted in this context

Figure 6: TEM images of aggregates formed from DSPE-PEG5k/PDLLA-MPEG mixtures with the mass ratio of DSPE-PEG5k to MPEG-PDLLA: (a) 0:1, (b) 0.0001:1, (c) 0.0005:1, (d) 0.001:1, (e) 0.005:1 and (f) 0.01:1.

Figure 7: TEM images of aggregates formed from DSPE-PEG1k/MPEG-PDLLA mixtures with the mass ratio of DSPE-PEG1k to MPEG-PDLLA: (a) 0:1, (b) 0.001:1, (c) 0.005:1, (d) 0.01:1, (e) 0.02:1 and (f) 0.03:1.

that less amounts of DSPE-PEG5k addition could effectively lead to a similar change of DSPE-PEG5k/PDLLA-MPEG morphology to that of DSPE-PEG2k/PDLLA-MPEG, while for DSPE-PEG1k, it needs more amounts of DSPE-PEG1k addition to gain the similar effect on the morphological transition. The intrinsic reason behind this phenomenon was due to the fact that the repulsive force between PEG segments of DSPE-PEG in the bilayer supramolecular structures formed by “ABA” temporary supra-amphiphiles and PDLLA-PEG increases with increasing PEG segment length of DSPE-PEG in the condition with the same number of “ABA” temporary supra-amphiphiles. As a result, the longer PEG segment of DSPE-PEG5k has a stronger repulsive force that can either maintain the existence of bilayer structure or destroy the structure of “ABA” temporary supra-amphiphiles at low PEG5k addition. In contrast, for DSPE-PEG1k/PDLLA-MPEG mixtures, the occurrence of morphological transition needs a higher DSPE-PEG1k addition because the shorter PEG segment has a less repulsive force. These obtained data further confirmed that the dynamic subtle balance between the number of “ABA” temporary supra-amphiphiles formed and the repulsive force strength between the PEG segments of DSPE-PEG under different DSPE-PEG additions induces the morphological transition of DSPE-PEG/PDLLA-MPEG complex aggregates.

4 Conclusions

In conclusion, the effects of adding DSPE-PEG to PDLLA-MPEG on the self-assembled structure were studied in this work. We have demonstrated that the self-assembled morphology of PDLLA-MPEG can be tuned via adding minor quantities of DSPE-PEG having two hydrophobic fatty acid tails. This unique geometric molecular structure of DSPE-PEG makes the DSPE segment to be preferentially interdigitated with the end of linear hydrophobic PDLLA segment of PDLLA-MPEG through the hydrophobic interaction. Thus, new “ABA” temporary supra-amphiphiles can be formed in which two fatty acid tails of DSPE-PEG can interact with three PDLLA segments of PDLLA-PEG. The as-formed new “ABA” temporary supra-amphiphiles tend to cooperatively assemble with PDLLA-MPEG into bilayer structure that is a key step to transform from micelles to vesicles. Although, in the bilayer structure, all the hydrophobic chains are fully contacted to facilitate the formation of a thermodynamically stable supramolecular structure, the PEG segments of DSPE-PEG forming as an inner hydrophilic layer also produce a repulsion force between them, which may affect the stability of “ABA” temporary supra-amphiphiles and thus destroy the bilayer structure. Upon increasing the ratios of DSPE-PEG/PDLLA-MPEG, the number of this “ABA” temporary supra-amphiphile formed

and the repulsive force strength between the PEG segments of DSPE-PEG in the formed supramolecular structures both increase. Accordingly, the dynamic subtle balance between these two conflict factors in forming stable supramolecular structures at different DSPE-PEG additions leads the morphological structure of DSPE-PEG/PDLLA-MPEG complex aggregates to gradually transit from micelles to vesicles, semi-vesicles and then to mixed micelles with increasing ratios of DSPE-PEG/PDLLA-MPEG. Thus, these findings demonstrated that the DSPE-PEG2k addition can be effectively used to adjust the morphological transition of PDLLA-MPEG self-assembled structure. This simple and effective method may provide a new avenue to control the self-assembly of amphiphilic blocks into supramolecular structures with various morphologies.

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References

- (1) Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. *Adv Drug Delivery Rev.* 2001;47:113–31.
- (2) Rösler A, Vandermeulen GWM, Klo HA. Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. *Adv Drug Delivery Rev.* 2012;64:270–9.
- (3) Mai Y, Eisenberg A. Self-assembly of block copolymers. *Chem Soc Rev.* 2012;41:5969–85.
- (4) Wang XS, Guérin G, Wang H, Wang YS, Manners I, Winnik MA. Cylindrical block copolymer micelles and co-micelles of controlled length and architecture. *Science.* 2007;317:644–7.
- (5) Discher DE, Eisenberg A. Polymer vesicles. *Science.* 2002;297:967–73.
- (6) Pochan DJ, Chen ZY, Cui HG, Hales K, Qi K, Wooley KL. Toroidal triblock copolymer assemblies. *Science.* 2004;306:94–97.
- (7) Holder SJ, Sommerdijk NAJM. New micellar morphologies from amphiphilic block copolymers: disks, toroids and bicontinuous micelles. *Polym Chem.* 2011;2:1018–28.
- (8) Sun H, Liu DQ, Du JZ. Nanobowls with controlled openings and interior holes driven by the synergy of hydrogen bonding and π - π interaction. *Chem Sci.* 2019;10:657–64.
- (9) Nishiyama N, Kataoka K. Nanostructured devices based on block copolymer assemblies for drug delivery: designing structures for enhanced drug function. *Adv Polym Sci.* 2006;193:67–110.
- (10) Yildirim T, Traeger A, Sungur P, Hoeppener S, Kellner C, Yildirim I, et al. Polymersomes with endosomal pH-induced vesicle-to-micelle morphology transition and a potential application for controlled doxorubicin delivery. *Biomacromolecules.* 2017;18:3280–90.
- (11) Venkataraman S, Hedrick JL, Ong ZY, Yang C, Ee PLR, Hammond PT, et al. The effects of polymeric nanostructure shape on drug delivery. *Adv Drug Delivery Rev.* 2011;63:1228–46.
- (12) Blanazs A, Madsen J, Battaglia G, Ryan AJ, Armes SP. Mechanistic insights for block copolymer morphologies: how do worms form vesicles? *J Am Chem Soc.* 2011;133:16581–7.
- (13) Rikken P, Engelkamp H, Nolte R, Maan J, Van Hest J, Wilson D, et al. Shaping polymersomes into predictable morphologies via out-of-equilibrium self-assembly. *Nat Commun.* 2016;7:12606.
- (14) Reuther JF, Siriwardane DA, Campos R, Novak BM. Solvent tunable self-assembly of amphiphilic rod-coil block copolymers with chiral, helical polycarbodiimide segments: polymeric nanostructures with variable shapes and sizes. *Macromolecules.* 2015;48:6890–9.
- (15) Bai Y, Fan XD, Tian W, Liu TT, Yao H, Yang Z, et al. Morphology transitions of supramolecular hyperbranched polymers induced by double supramolecular driving forces. *Polym Chem.* 2015;6:732–7.
- (16) Cai CH, Wang LQ, Lin JP, Zhang X. Morphology transformation of hybrid micelles self-assembled from rod-coil block copolymer and nanoparticles. *Langmuir.* 2012;28:4515–24.
- (17) Hickey RJ, Haynes AS, Kikkawa JM, Park SJ. Controlling the self-assembly structure of magnetic nanoparticles and amphiphilic block-copolymers: from micelles to vesicles. *J Am Chem Soc.* 2011;133:1517–25.
- (18) Liu W, Mao J, Xue YH, Zhao ZL, Zhang HS, Ji XL. Nanoparticle loading induced morphological transitions and size fractionation of coassemblies from PS-*b*-PAA with quantum dots. *Langmuir.* 2016;32:7596–605.
- (19) Salim NV, Guo QP. Multiple vesicular morphologies in AB/AC diblock copolymer complexes through hydrogen bonding interactions. *J Phys Chem B.* 2011;115:9528–36.
- (20) Dobrosielska K, Takano A, Matsushita Y. Nanophase-separated structures of AB block copolymer/C homopolymer blends with complementary hydrogen-bonding interactions. *Macromolecules.* 2010;43:1101–7.
- (21) Russell TP, Jerome R, Charlier P, Foucart M. The microstructure of block copolymers formed via ionic interactions. *Macromolecules.* 1988;21:1709–17.
- (22) Xu GK, Feng XQ, Li Y. Self-assembled nanostructures of homopolymer and diblock copolymer blends in a selective solvent. *J Phys Chem B.* 2010;114:1257–63.
- (23) Joralemon MJ, McRae S, Emric T. PEGylated polymers for medicine: from conjugation to self-assembled systems. *Chem Commun.* 2010;46:1377–93.
- (24) Prencipe G, Tabakman SM, Welscher K, Liu Z, Goodwin AP, Zhang L, et al. PEG branched polymer for functionalization of nanomaterials with ultralong blood circulation. *J Am Chem Soc.* 2009;131:4783–7.
- (25) Figg CA, Carmean RN, Bentz KC, Mukherjee S, Savin DA, Sumerlin BS. Tuning hydrophobicity to program block copolymer assemblies from the inside out. *Macromolecules.* 2017;50:935–43.
- (26) Li S, He JL, Zhang MZ, Wang HR, Ni PH. Multicompartment morphologies self-assembled from fluorinated ABC triblock

- terpolymers: the effects of flexible and rigid hydrophobic moieties. *Polym Chem.* 2016;7:1773–81.
- (27) Singer SJ, Nicolson GL. The fluid mosaic model of the structure of cell membranes. *Science.* 1972;175:720–31.
- (28) Simons K, Ikonen E. Functional rafts in cell membranes. *Nature.* 1997;387:569–72.
- (29) Le Meins JF, Schatz C, Lecommandoux S, Sandre O. Hybrid polymer/lipid vesicles: state of the art and future perspectives. *Mater Today.* 2013;16:397–402.
- (30) Peyret A, Ibarboure E, Le Meins JF, Lecommandoux S. Asymmetric hybrid polymer–lipid giant vesicles as cell membrane mimics. *Adv Sci.* 2018;5:1700453.
- (31) Hinman SS, Ruiz CJ, Cao Y, Ma MC, Tang JJ, Laurini E, et al. Mix and match: coassembly of amphiphilic dendrimers and phospholipids creates robust, modular, and controllable interfaces. *ACS Appl Mater Interfaces.* 2017;9:1029–35.
- (32) Tuyen Dao TP, Brûlet A, Fernandes F, Er-Rafik M, Ferji K, Schweins R, et al. Mixing block copolymers with phospholipids at the nanoscale: from hybrid polymer/lipid wormlike micelles to vesicles presenting lipid nanodomains. *Langmuir.* 2017;33:1705–15.
- (33) Zhai JL, Hinton TM, Waddington LJ, Fong C, Tran N, Mulet X, et al. Lipid-PEG conjugates sterically stabilize and reduce the toxicity of phytantriol-based lyotropic liquid crystalline nanoparticles. *Langmuir.* 2015;31:10871–80.
- (34) Liu Z, Sun XM, Nakayama-Ratchford N, Dai HJ. Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery. *ACS Nano.* 2007;1:50–56.
- (35) Xiao MY, Xia GJ, Wang R, Xie DQ. Controlling the self-assembly pathways of amphiphilic block copolymers into vesicles. *Soft Matter.* 2012;8:7865–74.
- (36) Prhashanna A, Tan WK, Khan SA, Chen SB. Co-micellization behavior of triblock copolymers in the presence of hydrophobic drug molecules: a simulation study. *Colloids Surf B.* 2016;148:299–307.
- (37) Prhashanna A, Khan SA, Chen SB. Co-micellization behavior in poloxamers: dissipative particle dynamics study. *J Phys Chem B.* 2015;119:572–82.
- (38) Hafezi MJ, Sharif F. Brownian dynamics simulation of co-micellization of amphiphilic block copolymers with different tail lengths. *Langmuir.* 2012;28:16243–16253.