Construct Biomimetic Giant Vesicles via Self-Assembly of Poly(2-methacryloyloxyethyl phosphorylcholine)-*block*-poly(D,L-lactide)

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ABSTRACT: The poly(2-methacryloyloxyethyl phosphorylcholine)-*block*-poly(D,L-lactide) (PMPC-*b*-PLA) was specially designed to develop biomimetic giant vesicles (GVs) and giant large compound vesicles via a simple spontaneous assemble in aqueous solution. The weight fraction of the hydrophilic PMPC block (f_{PC}) was proved to play an important role in the size and morphology control of the self-assembled aggregates. The GVs with controlled micrometer size and biomimetic PMPC corona have great potential as artificial cell models. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 3197–3202, 2010

Key words: biomimetic; block copolymers; self-assembly; vesicles

INTRODUCTION

Vesicles self-assembled from lipids or polymeric amphiphilic copolymers have attracted great attention as useful model for artificial cells owing to their micrometer size, hollow and lamellar spherical structure.[1–6] Compared to liposome, polymer vesicles possess highlighted distinctive properties, such as good stability and permeability.[7,8] A decade ago, Discher et al. first reported a type of giant vesicles (GVs) formed from poly(ethylene oxide)-*block*-poly(ethylethylene) (PEO-*b*-PEE) having a similar hydrophilic fraction (35% ± 10%) to liposomes, and these vesicles were termed polymersomes.[9] Zhou and Yan further developed a kind of polymer GVs with sizes controlled by ill-defined hyperbranched copolymers[10] and monitored real-time shape transformations of vesicles to mimic cellular fission and fusion.[3,4] Recently, GVs with controllable size in microns and excellent stability prepared from synthetic polypeptides and graft copolymers have also been reported.[11,12] These polymeric GVs were proved that they provided lots of new possibility to mimic cellular processes.

Phosphorylcholine (PC) is an interesting kind of zwitterionic molecular segment, which is present at the end of the lipid and on the external surface of cell membrane. Phosphorylcholine (PC)-based polymers such as poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) have been proven to improve biocompatibility and prevent nonspecific protein adsorption on the macroscopic biomaterials.[13–15] Recently, we and others have proposed that PC-based functional molecules and polymers can be used for surface modification of various nanomaterials.[16–18] Similar to PEO, the biocompatible and hydrophilic PMPC blocks were also used in preparing nanoparticles with excellent cytocompatibility. For example, nanometer-sized micelles and vesicles with PMPC corona have been prepared, and these assemblies were considered as effective vehicles for drug and DNA delivery.[19–22] More interestingly, the zwitterionic "lipid-like" molecule was reported to present very unique cell uptake ability, which has been ascribed to the same possibility of fusion with cell membrane as liposome.[23]

Although the development of PC-based polymer is inspired by the surface structure of a cell membrane, the available research of its assemblies in aqueous solutions is limited in the nanoscale and unsuitable as plausible models for artificial cells. We

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TABLE I
Details of the PMPC-*b*-PLA Copolymers and Vesicles

Copolymer	Molar ratio in feed PLA-Br : MPC	M_n[a] (g mol^{-1})	f_{PC}[a]	PDI[b]	Morphology	Diameter[c] (μm)
$PMPC_{21}$-*b*-PLA_{73}	1 : 25	11,800	0.53	1.18	Giant vesicles	3.5 ± 1.6
$PMPC_{17}$-*b*-PLA_{73}	1 : 20	10,600	0.48	1.25	Giant vesicles	11.0 ± 3.9
$PMPC_{12}$-*b*-PLA_{73}	1 : 15	9100	0.40	1.33	Large compound vesicles	15.0 ± 9.4

[a] Determined by ^{1}H-NMR.
[b] The PDI of PMPC-*b*-PLA was determined by using dimethylformamide as the elution solvent.
[c] Measured from the microscopy studies, and the results were based on analysis of 200 vesicles for each sample.

hypothesize here that polymer vesicles with PMPC corona will be surely interesting as artificial cell models because of the biomimetic structure and micrometer size. Herein, the poly(2-methacryloyloxyethyl phosphorylcholine)-*block*-poly(D,L-lactide) (PMPC-*b*-PLA) with short hydrophilic PMPC blocks was specially designed to develop biomimetic GVs via an easy solvent-injection method.[24] The effect of the weight fraction of PMPC (f_{PC}) onto the assembly morphology was investigated with a goal to develop plausible models for artificial cells.

EXPERIMENTAL

Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized according to the literature.[25] The PMPC-*b*-PLA diblock copolymers used in this study were synthesized by atom transfer radical polymerization (ATRP) of MPC monomer using PLA-Br as macroinitiator. The details of synthesis procedure can be found elsewhere.[19] The M_n value and chemical structure of PMPC-*b*-PLA were confirmed by ^{1}H-NMR spectra (500 HZ). Molecular weights and their distributions were determined by GPC. The elution solvent was dimethylformamide, and the flow rate was 1 mL min^{-1} (40° C).

Preparation of PMPC-*b*-PLA vesicles in aqueous solution

Fifty milligrams of PMPC-*b*-PLA copolymers were first dissolved in the mixture of methanol and chloroform (1 : 1, v/v). Then, 50 μL of the obtained solution was injected into deionized water and stirred vigorously at room temperature for 2 h. Dialysis was performed against deionized water for 2 h to remove methanol. Chloroform was completely removed by evaporation at 4°C overnight. All PMPC-*b*-PLA solutions were stored at 4°C to minimize degradation.

Optical and fluorescence microscopy

Olympus IX71 inverted fluorescence microscope with a 100× oil objective was used to visualize PMPC-*b*-PLA vesicles at room temperature. Fluorescent labeling of vesicles was performed by simply adding a drop of Rhodamine 6G/water solution (0.2 mg mL^{-1}) into the PMPC-*b*-PLA vesicle aqueous solution (1 mL) and vortexed for 30 s. Then, the sample was directly observed by fluorescence microscopy equipped with a color video recorder.

Transmission electron microscopy

For transmission electron microscopy (TEM) measurement, a drop of the vesicle solution was placed onto a carbon-coated grid, washed with a negative stain solution (2% uranyl acetate solution), and blotted with a filter paper. The specimens were observed with a TEM (JEM 1230, JEOL).

Scanning electron microscopy

The vesicle solution was dropped onto freshly cleaved mica for scanning electron microscopy (SEM) measurement. After being coated with gold, the sample was observed using a SEM (Sirion-100, FEI).

Dynamic light scattering measurements

The average diameter and size distribution of aggregates were measured using a laser particle-size analyzing system (Brookhaven 90 plus, Brookhaven Instruments Corporation) at the scattering angle of 90°.

RESULTS AND DISCUSSION

PMPC-*b*-PLA diblock copolymers were synthesized by ATRP as reported previously.[19] The molecular weight of PLA-Br used in this study was 5500 g mol^{-1} determined by ^{1}H-NMR. The feeding molar ratio was specially designed to synthesize PMPC-*b*-PLA with a short hydrophilic PMPC block as displayed in Table I. The subscripts of the copolymer denote the number-average degrees of polymerization of each block. The f_{PC} in Table I represents weight fraction of the hydrophilic PMPC block.

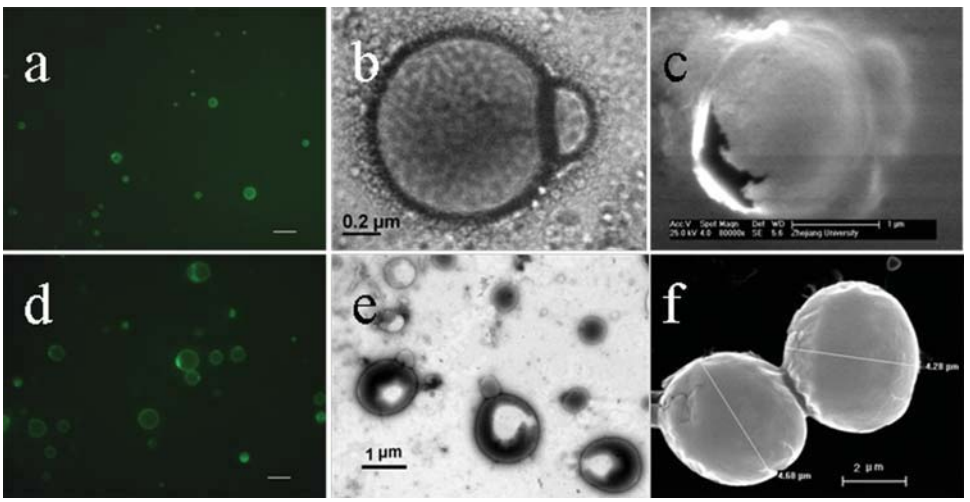

Figure 1 Fluorescent micrographs (a, d), TEM (b, e), and SEM (c, f) images of PMPC-b-PLA giant vesicles. (a), (b), and (c) are PMPC21-b-PLA73 vesicles; (d), (e), and (f) are PMPC17-b-PLA73 vesicles. The scale bars represent 10 μm in (a) and (d). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Characterization of PMPC-*b*-PLA giant vesicles

When the f_{PC}s are 0.53 and 0.48, which correspond to $PMPC_{21}$-*b*-PLA_{73} and $PMPC_{17}$-*b*-PLA_{73}, it is of interest to find both of them spontaneously aggregate into GVs (showed in Fig. 1). Figure 1(a,d) is the fluorescent micrographs of the resulting self-assembled objects. The hollow interior of the vesicular morphology is clearly illustrated as evidenced by a significant decrease in fluorescence intensity toward the center of the spheres. It appears that assemblies formed from $PMPC_{21}$-*b*-PLA_{73} and $PMPC_{17}$-*b*-PLA_{73} both have a well-defined vesicular structure and a micrometer size, which is in the range of 1–10 μm. In representative TEM images of Figure 1(b,e), the hollow structure of the vesicles can also be deduced from the increased blackness caused by the staining agent.[26–29] GVs are further studied by SEM [Fig. 1(c,f)] and show a collapsed and three-dimensional sphere structure, consistent with the TEM results. In addition, the vesicle size measured by TEM and SEM is smaller than that scaled by fluorescent microscopy because of shrinkage and collapse of dry vesicles. In fact, vesicles can also be obtained by directly dissolving diblock copolymer into water [Fig. 2(a)]. Therefore, the formation of the vesicle can be further evidenced by their ^{1}H-NMR spectra in D_2O. Different from that measured in the mixture of methanol-*d*4 and *d*-chloroform (1 : 1, v/v), all the peaks related to PLA disappear, whereas all peaks of PMPC are still remaining [Fig. 2(b)]. These imply that during the process of PMPC-*b*-PLA GV formation, the hydrophobic PLA blocks tend to associate with each other to minimize direct exposure to water, whereas the hydrophilic PMPC blocks face inner and outer hydrating solutions, and thereby delimit the two interfaces of a typical sandwich-like membrane. Based on the above results, the aggregates appear to be GVs with hollow, micrometer-size and three-dimensional spheres.

Usually, there is a general empirical law which stipulates that block copolymers possessing a hydrophilic weight fraction (f) > 0.45 are expected to form spherical micelles, whereas those with f around 0.35 ± 0.1 are predicted to form polymeric vesicles.[1] However, the data here indicated that the critical f_{PC} to form polymeric vesicles in PMPC-*b*-PLA system are higher than that of 0.35 ± 0.1, which might be ascribed to the unique zwitterionic side-chain structure of MPC. In fact, spherical micelles were also obtained when $f_{PC} > 0.6$.

Controllable size of the PMPC-*b*-PLA GVs

To explore polymeric GVs for many potential applications such as good models for the simulation of the fluidization of cell membranes,[30] controlled size of vesicle is necessary. Dynamic light scattering (DLS) result shows that the size of $PMPC_{21}$-*b*-PLA_{73} vesicles has two fractions [Fig. 3(a)]. One type of vesicles presents sizes larger than 1 μm, which make up most of the total. These vesicles should be that showed in Figure 1(a). The other vesicles have nano-size around 100 nm. In fact, nano-vesicles are usually obtained when preparing GVs (data not shown). In Figure 1(d), most of $PMPC_{17}$-*b*-PLA_{73} vesicles have a size larger than 5 μm, which is not suitable for DLS analyzing. To compare the sizes of the two GVs, the size distributions of PMPC-*b*-PLA vesicles are measured by artificially analyzing 200 GVs from the staff gauge in the fluorescent micrographs for

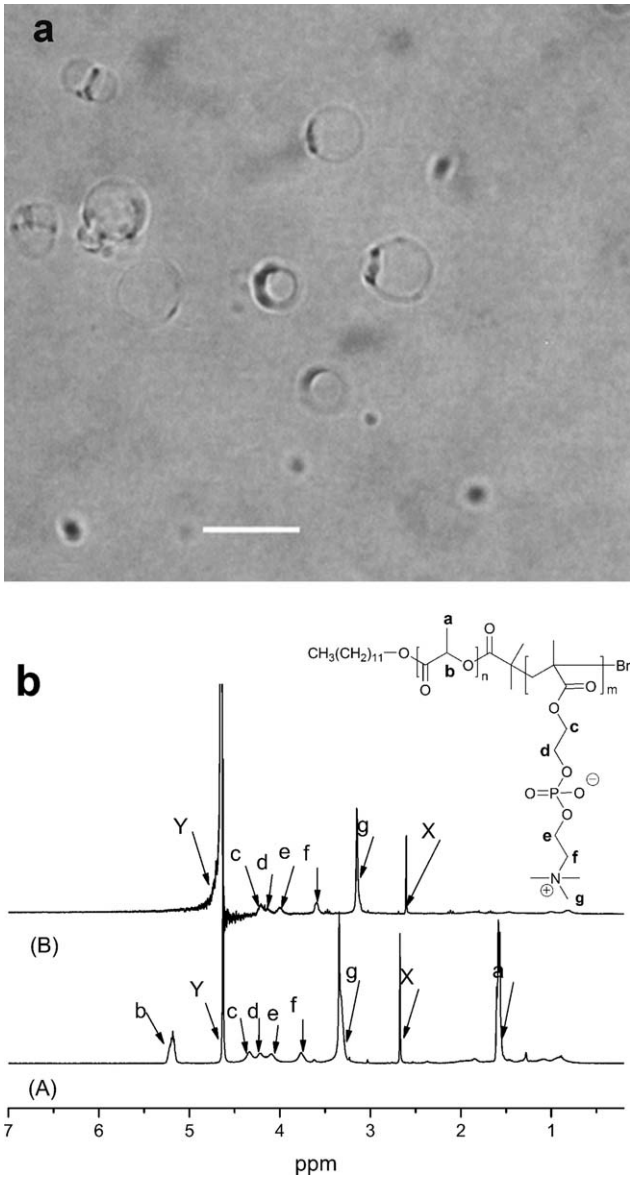

Figure 2 (a) Optical micrograph of giant vesicles prepared by directly dissolving diblock copolymer into water. (b) ^{1}H-NMR spectra and their assignments of PMPC-*b*-PLA in $CDCl_3/CD_3OD$ (A) and D_2O (B). *X* and *Y* are peaks of DMSO and H_2O.

each sample [displayed in Fig. 3(b)]. This method has been adopted by other groups for comparing sizes of GVs.[10,12] The average diameters of the vesicles are 3.5 ± 1.6 μm (closed to DLS result) for $PMPC_{21}$-*b*-PLA_{73} and 11.0 ± 3.9 μm for $PMPC_{17}$-*b*-PLA_{73}. Thus, the size of the generated vesicles increases, and the size distribution becomes broader as the f_{PC} of the PMPC-*b*-PLA molecules decreases from 0.53 to 0.48, which indicate that the sizes of GVs can be controlled by their hydrophilic weight fraction and the block copolymers with shorter hydrophilic blocks assembled into larger vesicles. The weight fraction of hydrophilic block can present an easy approach to prepare polymer vesicles with well-controlled sizes, which has been reported by other groups.[10,31]

The $PMPC_{21}$-*b*-PLA_{73} and $PMPC_{17}$-*b*-PLA_{73} vesicles are comparatively stable and can be stored for at least 2 months in water at around 4°C. Compared with the polymer vesicles previously reported, the biomimetic membrane structure of PMPC corona and controlled micrometer size make this new type of aggregations more like artificial cells.

Characterization of giant large compound vesicles

We also synthesized $PMPC_{12}$-*b*-PLA_{73} diblock copolymers with an f_{PC} of 0.40. After injecting the copolymer solution into stirring water, large compound vesicles (LCVs) with micrometer size were

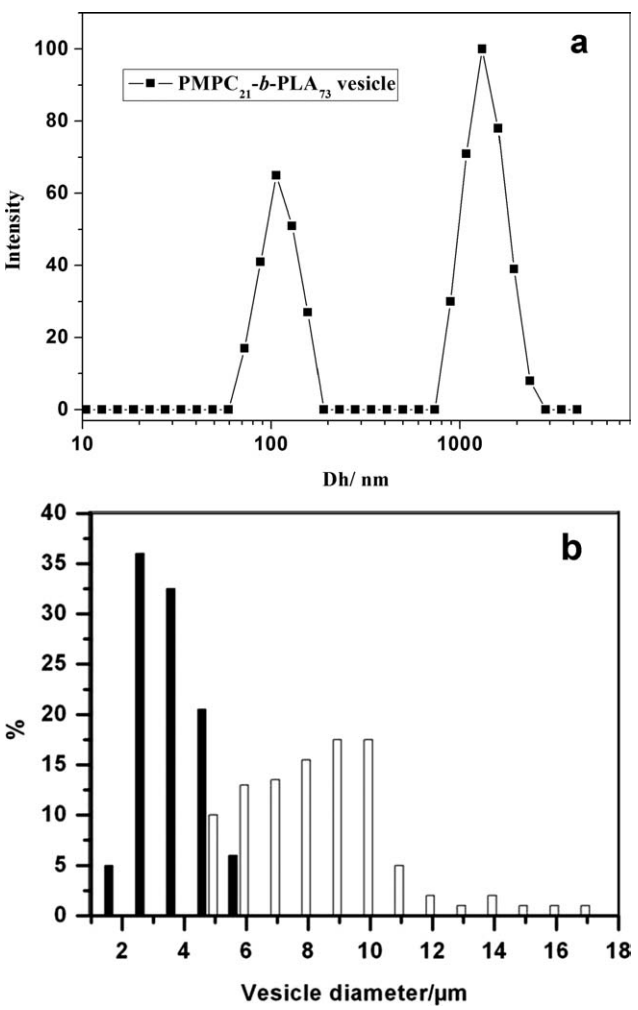

Figure 3 (a) Size distribution of PMPC21-*b*-PLA73 vesicles, determined by DLS; (b) size distribution of GVs based on artificially analyzing 200 giant vesicles from the fluorescent micrographs. The black and white bars represent PMPC21-*b*-PLA73 and PMPC17-*b*-PLA73 vesicles, respectively.

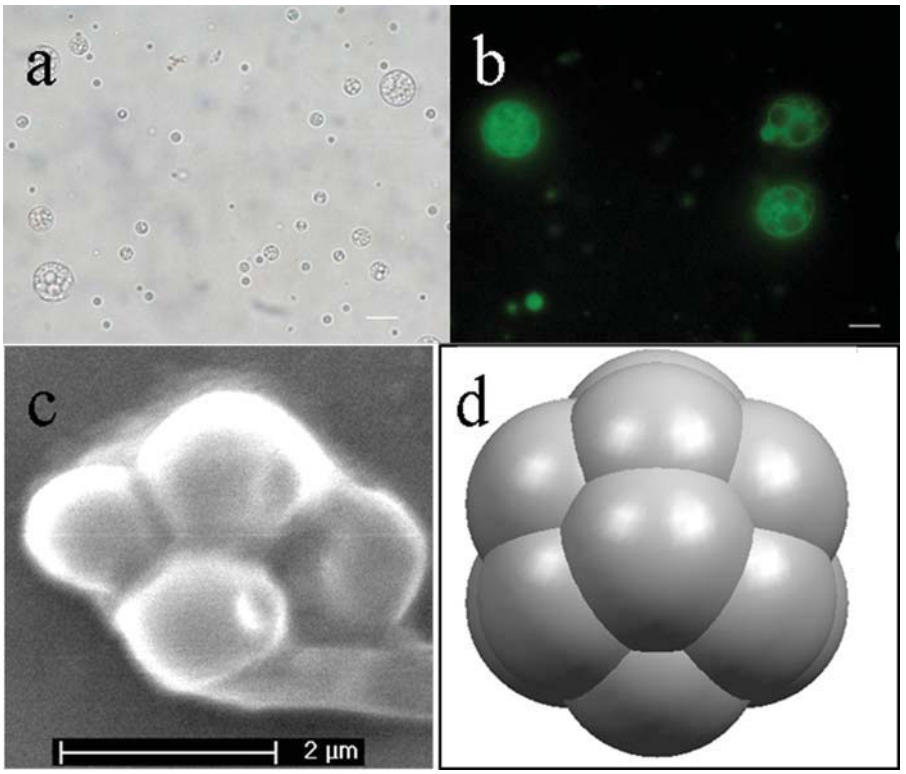

Figure 4 Optical and fluorescent micrographs (a, b), and SEM images (c) of PMPC15-b-PLA73 giant LCVs. (d) is the three-dimensional model of the LCVs. The scale bars represent 10 μm in (a) and (b). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

obtained, and they have clearly visible multicell internal structures (Fig. 4). The morphology of PMPC-*b*-PLA LCVs is quite similar to that of the LCVs reported before,[32–34] but the size is much larger. Figure 4(a,b) shows the individual vesicles of the LCVs all have a hollow interior; while the SEM images of Figure 4(c) indicate the three-dimensional honeycomb-like structure of LVCs with many individual vesicles tightly adhering together. Figure 3(d) is the three-dimensional model of the LCVs. As LCVs are complex aggregates based on the individual vesicles, the "vesicle aggregation number," n, is investigated as an important character [Fig. 5(a)]. The result exhibits the relative population of the LCVs, where $n > 4$ makes up more than 80% of the total. Figure 5(b) displays that the average diameter of the LCVs is 15.0 ± 9.4 μm, and the size distribution ranges from 5 to 30 μm. Mai et al.[35] have reported that the formation of the LCVs actually involves a secondary aggregation of individual vesicles and a subsequent

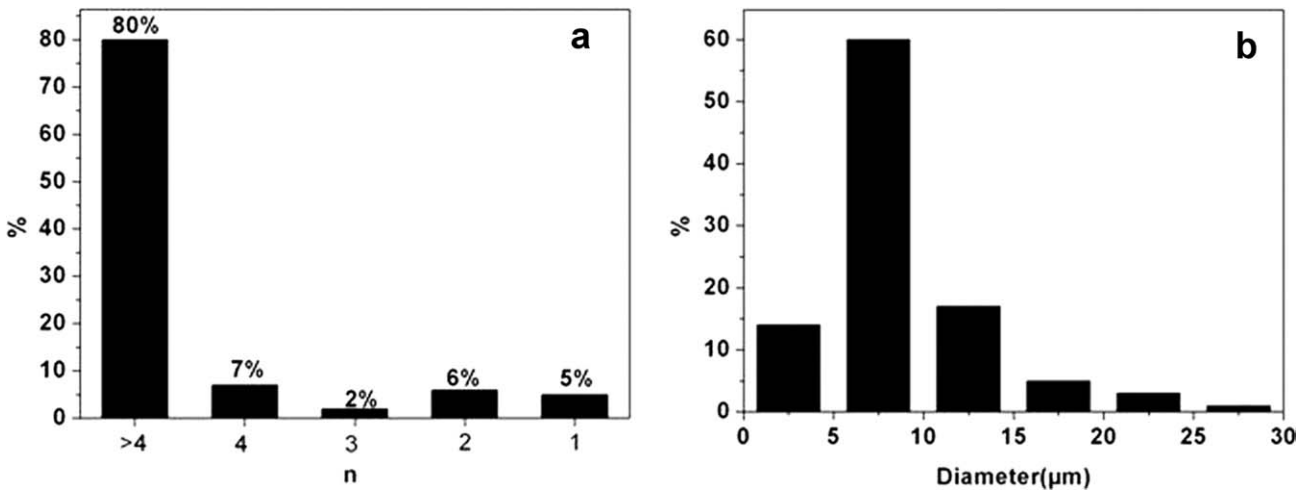

Figure 5 (a) Population distribution of the LCVs, and (b) size distribution of the LCVs.

fusion process. Different from $PMPC_{21}$-*b*-PLA_{73} and $PMPC_{17}$-*b*-PLA_{73} diblock copolymers, $PMPC_{12}$-*b*-PLA_{73} that we synthesized has the shortest hydrophilic blocks. When the copolymers self-assembled in aqueous solution, we consider that they first aggregated into individual GVs. However, the short PMPC blocks are not hydrophilic enough to stabilize the individual vesicles and then the vesicles tend to undergo adhesion and fusion to form LCVs. The results also indicate that f_{PC} plays an important role in self-assembly morphology of PMPC-*b*-PLA diblock polymers. Some features of PMPC-*b*-PLA LCVs such as biocompatible PMPC corona, degradable vesicle wall, and micrometer size may offer the giant LCVs an admirable model system for studying the real-time drug release behavior of multivesicular aggregates.

CONCLUSIONS

In conclusion, we successfully synthesize a series of PMPC-*b*-PLA diblock copolymers with short f_{PC}. By directly injecting the copolymer solution into water, it is very interesting to find that these diblock copolymers actually spontaneously self-assemble into GVs and giant LCVs. The weight fraction of PMPC (f_{PC}) is proved to play an important role in the size and morphology control of the self-assemble aggregates. The micrometer size and the biomimetic membrane structure of PMPC corona make the GVs and LCVs can be as useful models for artificial cells and studying the real-time drug release.

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