PC-Polyolefins: Synthesis and Assembly Behavior in Water

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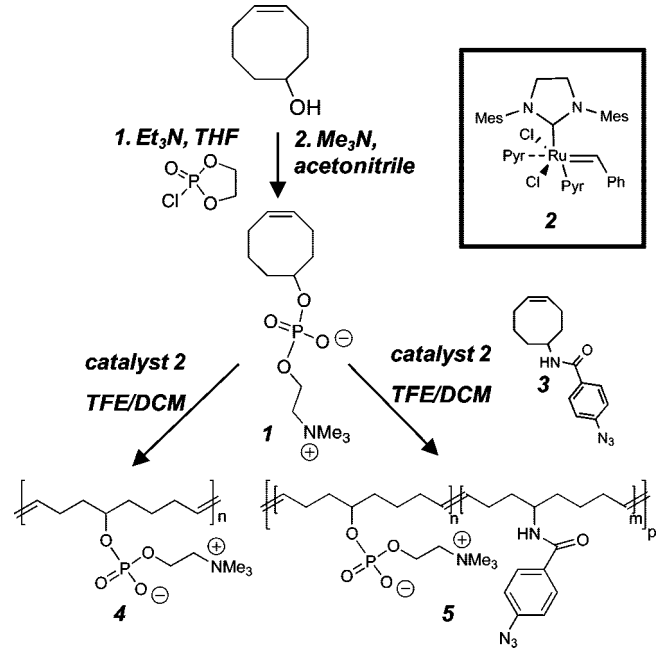
Received March 26, 2009
Revised Manuscript Received April 2, 2009

The hydrophilic and biocompatible properties of phosphorylcholine (PC)-based polymers enable their use as antifouling coatings and in blood-contacting medical implants and devices.[1] Prominent among PC-polymers are those prepared from the monomer methacryloyloxyethylphosphorylcholine (MPC).[2] MPC homopolymers, as well as their copolymers containing methacrylic comonomers, are prepared by conventional free radical polymerization[3] or by controlled free radical techniques that afford well-defined polymers with tailored end groups.[4]

Phosphorylcholine and phosphatidylcholine amphiphiles undergo self-assembly into bilayer liposomes that mimic natural cell membranes.[5] These pH and temperature tunable structures can be used in drug delivery, by carrying and releasing encapsulated hydrophilic cargo, and/or hydrophobic molecules embedded within the bilayer.[6] One limitation of conventional liposomes pertains to their lack of robust mechanical properties. Thus, incorporation of polymers, such as polyMPC and poly(ethylene glycol) (PEG), and/or reactive cross-linkers, into the bilayer can increase stability and mechanical integrity.[7,8] Alternatives to conventional small molecule liposomes are diblock copolymer-based structures termed polymersomes.[9−11] While diblock-based polymersomes are considerably tougher (having cohesive energy density, E_C, ~2.2 mJ/m^2) than small molecule liposomes, their formation depends critically on having precise volume fractions of the respective blocks. Interestingly, although the PC functional group of polyMPC mimics natural phospholipids, polyMPC is too hydrophilic to possess the inherent self-assembly capabilities observed in small molecule phospholipid amphiphiles. Integration of hydrophobic monomers[12] or end groups[13] into the structure is needed for polyMPC to form polymersomes. For other polymers, such as poly(ethylene oxide)-*block*-poly(ethylethylene) (PEO−PEE) polymersomes, tuning the weight fraction of each block enables their assembly into different architectures, including rodlike micelles and vesicles. These polymers are biocompatible and able to encapsulate various small molecules and proteins, making them interesting delivery vehicles for polymer therapeutics.[14]

Here we describe the synthesis of phosphorylcholine-substituted polyolefins (which we term "PC-polyolefins") by ring-opening metathesis polymerization (ROMP) and their aqueous assembly into polymersome structures. As shown in Scheme 1, 5-phosphorylcholine cyclooctene (PC-COE) (**1**) was prepared by reacting 5-hydroxycyclooctene with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) in dry tetrahydrofuran (THF), followed by ring-opening with trimethylamine in dry acetonitrile. Lyophilization gave the desired PC monomer **1** as a white powder in 60% yield, which by NMR spectroscopy showed characteristic cyclooctene alkene signals (^{1}H at 5.25 ppm and ^{13}C at 129.1 and 128.7 ppm) and the expected signal at 0 ppm in the ^{31}P spectrum. Fast atom bombardment (FAB) mass spectrometry gave one major signal at 292.168 g/mol (M + H$^+$). PC-monomer **1** was then used in ring-opening metathesis polymerization (ROMP) with the pyridine-substituted version of Grubbs' ruthenium benzylidene metathesis catalyst (H_2IMes)(Cl)$_2$(pyr)$_2$RuCHPh (**2**).[15] The functional group tolerance of ruthenium-based ROMP catalysts allows not only for homopolymerization of **1** but also for copolymerization of **1** with other substituted cyclic olefins, such as phenylazide-substituted cyclooctene **3**[16] which integrates a photo-cross-linkable (nitrene-generating) group into the polymer backbone.

Scheme 1



PC-polyolefin homopolymer **4** and azide-containing PC-polyolefin **5** were obtained as white solids in >95% yield and characterized by gel permeation chromatography (GPC) and NMR spectroscopy. Aqueous GPC (using PEO calibration) performed on homogeneous aqueous polymer solutions (~5 mg/mL) gave number-average molecular weights (M_n) ranging from 3000 g/mol to greater than 100 000 g/mol, with polydispersities of 1.4−2.5, as shown in Table 1. A measure of molecular weight control was achieved by adjusting monomer-to-catalyst ratio up to M_n ~50 000 g/mol. NMR spectroscopy performed on PC-polyolefins **4** and **5** showed olefin signals at 5.17 ppm in the ^{1}H spectrum, and at 135.0 and 134.0 ppm in the ^{13}C spectrum, shifted as expected relative to the cyclic olefin signals of monomers **1** and **3**. Trans olefins dominated the backbone structure, as seen by IR (970 and 1280 cm^{-1}) and ^{13}C NMR spectroscopy, and the presence of azide functionality in polymer **5** was noted by IR (2121 cm^{-1}) as well.

These novel PC-substituted polymers, containing a PC group at every eighth backbone carbon atom on average, were found to possess a hydrophobic/hydrophilic balance appropriate for aqueous assembly into polymer vesicles, using film hydration techniques similar to those employed for conventional liposome formation from small molecule zwitterions.

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10.1021/ma900653h CCC: $40.75
Published on Web 04/15/2009

Table 1. Molecular Weight Characteristics of Polymers 4 (Entries 1−4) and 5 (Entries 5 and 6)

entry	**1**:**2**:**3**	theor M_n	M_n[a]	M_w[a]	PDI	mol % **3**[b]
1	25:1:0	7 275	5 000	11 000	2.2	0
2	100:1:0	29 100	23 000	51 000	2.3	0
3	200:1:0	58 200	72 800	98 400	1.4	0
4	250:1:0	72 750	92 000	127 000	1.4	0
5	85:1:15	28 785	7 400	17 200	2.3	15
6	95:1:5	28 995	7 400	13 800	1.9	5

[a] As determined by SEC on 5 mg/mL solutions. [b] As determined by ^{1}H NMR spectroscopy.

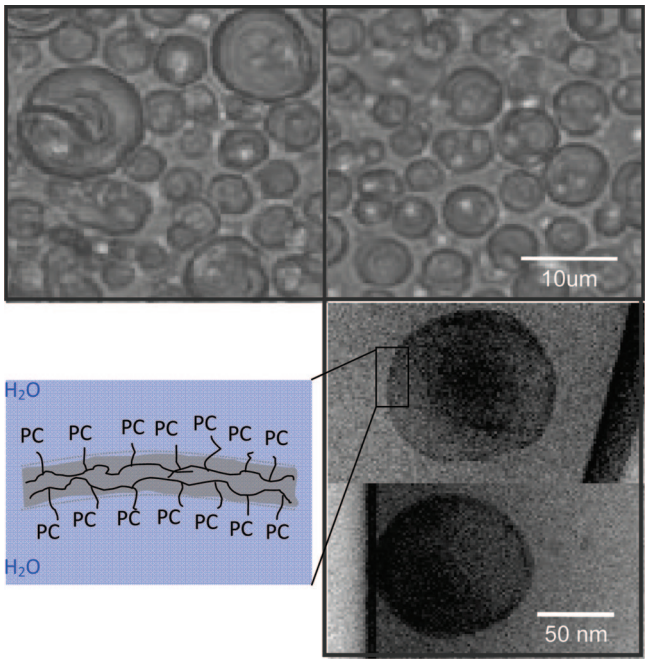

Figure 1. Top: bright field images of DLPC liposomes (left) and polymersomes from **4** (right). Bottom: schematic of PC-polyolefin assembly into a polymersome structure (left) and cryo-TEM images of a PC-polymersome from **5**.

In a typical procedure, a methanol/chloroform solution of the polymer (∼20 mg/mL) was dried to a thin film, hydrated with water (∼1 mL), and agitated at 400 rpm and 40 °C for 2 h. The resultant vesicle solutions were subjected to freeze−thaw cycles that afford large (∼1−5 μm diameter) vesicles, which were seen by optical microscopy to be comparable in size and shape to liposomes prepared from 1,2-dilauryl-*sn*-gylcero-3-phosphocholine (DLPC). Figure 1 (top) compares bright field micrographs of structures prepared from DLPC (left) and PC-polymer **4** (right).

These micron-diameter PC-polymer vesicles were reduced to smaller (<500 nm) structures by sonication, followed by passage through track-etch membranes (AvantiLipids, Inc.) having an average pore diameter of 50 nm. The small PC-polymer vesicles, prepared from polymers **4** and **5**, were characterized by cryo-transmission electron microscopy (cryo-TEM), exemplified in the cryo-TEM structures of Figure 1. In cases where the polymer vesicles were prepared from monomer **5**, a range of 5−15 mol % phenylazide was integrated into the structure, and the formed vesicles were subjected to irradiation at 302 nm for 1 min (120 000 μJ with a UVP CL1000). Figure 1 (bottom) shows examples of cryo-TEM images of small vesicles formed from PC-polyolefin **5**, prepared by vitrification of the samples on lacey carbon grids. These images confirm the presence of smaller vesicles following the extrusion step, which ranged from ∼50 nm (shown) up to 1 μm. That many observed vesicles from polymers **4** and **5** are much larger than the diameter of the track-etch membrane pores suggests qualitatively that these vesicles are more robust than typical liposomes and that they tend to resist "downsizing" associated with extrusion.[17] This tough but elastic nature of the structures is, we speculate, a consequence of the polymer composition, and its comblike architecture in which the hydrophobic portion must extend along (parallel to) the membrane, rather than across (perpendicular to) the membrane. Cryo-TEM images allow clear visualization of the very thin (<5 nm thick) membrane. In the cross-linked cases, the images suggest that vesicles have become trapped within vesicles, a result of incomplete formation of the vesicular structure.

In summary, novel PC-polyolefin polymers and copolymers were prepared by ROMP of PC-substituted cyclooctene and found to exhibit interesting aqueous assembly behavior that is potentially useful in various modes of encapsulation and delivery. These PC-polyolefin homopolymers and random copolymers possess an intrinsic hydrophobic/hydrophilic balance needed to form polymersomes, which occurred readily over a wide range of homopolymer and copolymer molecular weights. These structures are thus distinct from polymersomes formed from block copolymer structures,[9−12] which have narrow molecular weight distribution or tightly controlled comonomer ratios. PC-polyolefin vesicles are qualitatively more robust than conventional liposomes, providing potential advantages in delivery systems. Further evaluation of the physical properties and permeability of these structures is under investigation and will be reported subsequently.

Acknowledgment. Financial support from the National Science Foundation (CBET-0553597 and the Materials Research Science & Engineering Center at UMass Amherst) and the Army Research Office (MURI award) is acknowledged with thanks.

Supporting Information Available: Synthetic and assembly procedures and spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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MA900653H