Synthesis, Characterization and Micellization of Heterograft Copolymers Based on Phosphoester Functionalized Macromonomers via "Grafting Through" Method

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ABSTRACT: Novel amphiphilic heterograft copolymers consisting of phosphoester functionalized PEG (phosPEG) and PCL (phosPCL) were synthesized by the ring-opening polymerization via "grafting through" method. The heterograft structure and thermal properties of these copolymers with various compositions were characterized by ^{1}H-NMR, ^{31}P NMR, size exclusion chromatography (SEC), and differential scanning calorimetry (DSC) in detail. These amphiphilic copolymers could self-assemble into micellar structures in aqueous solution, and their critical micellization concentrations (CMC) were determined to be 0.69–1.25 mg/L by fluorescence technique. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements show that these heterograft copolymer micelles are spherical in shape with the particle size ranging from 20 to 60 nm, which has potential in biomedical application. J Appl Polym Sci 123: 365–374, 2012

Key words: heterograft copolymer; grafting through; PCL; PEG; phosphoester

INTRODUCTION

Over the past two decades, polymers with complex architectures such as block, star, graft, hyperbranched, dendrimer, and cyclic structures have been extensively investigated due to the rapid development of controlled/living polymerization techniques.[1–3] Graft copolymers, also called as molecular brushes, have attracted considerable interest for their distinguished conformation and properties.[3–10] Heterograft copolymers are molecular brushes with intermixed side chains of more than one identity and have exhibited specific properties in crystallization and micellization relating to their variety of compositions.[3] Neugebauer et al. synthesized the heterograft brushes with poly(ethylene glycol) methyl ether methacrylate (PEOMA) and octadecyl methacrylate (ODMA) or octadecyl acrylate (ODA) side chains [PEOMA/ODMA, PEOMA/ODA] by atom transfer radical polymerization (ATRP) and investigated the crystallization behaviors of these copolymers.[11] Jérôme et al. reported the synthesis of heterograft brushes obtained from the copolymers of ε-caprolactone and α-chloro-ε-caprolactone by combination of atom transfer radical addition (ATRA) and ATRP techniques.[12] Huang et al. prepared heterograft copolymers of poly(-GTEMPO-*co*-EO)-*g*-PS/P*t*BA in one-pot by atom transfer nitroxide radical coupling (ATNRC) reaction and studied the graft efficiency.[13] Other structures of heterograft copolymers have also been reported.[14,15]

There are three main strategies to prepare graft (co)polymers: "grafting onto," "grafting from," and "grafting through."[1–3] The "grafting through" method involves the synthesis of macromonomers with terminal functionality. The most attractive advantage of this method is that the side chains can be characterized prior to polymerization, which allows the preparation of brushes with well-defined grafting density and side-chain length.[3] However, this strategy is difficult to proceed due to the reasonably low concentration of polymerizable end groups and high steric hindrance of the propagating chain end.[3,5,6] Several polymerizing mechanisms have been proven accessible to this method, including anionic,[16] free radical,[6,9,11] and ring-opening metathesis.[17] To the best of our knowledge, "grafting through" method by ring-opening polymerization (ROP) has been rarely reported.

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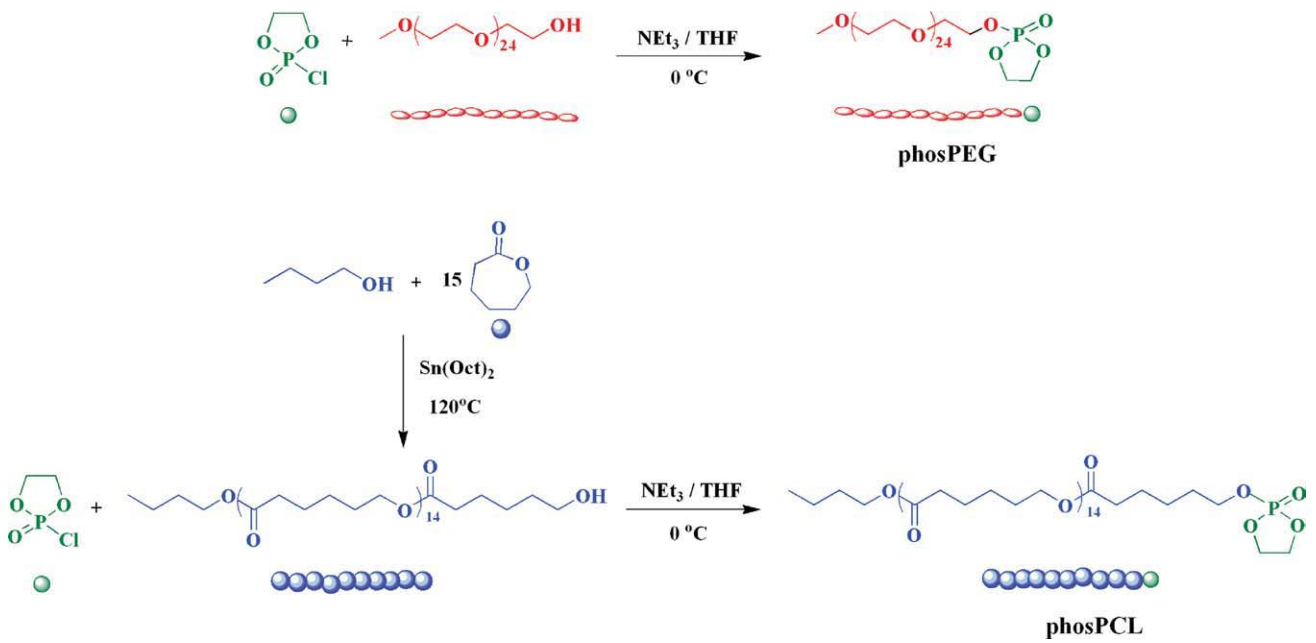


Scheme 1 Synthetic routes of cyclic phosphoester functionalized macromonomers (phosPEG and phosPCL) [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.].

Recently, polyphosphoesters have been interested for biological and pharmaceutical applications owing to their favorable biocompatibility and biodegradability.[18–20] Penczek et al. prepared polyphosphoesters as carriers of cations in processes of biomineralization.[19] Zhuo et al. reported the poly/copolymerization of a series of five-membered cyclic phosphates showing the potential for drug delivery.[21–23] Leong et al. studied the application of polyphosphoesters in the fields of drug delivery, tissue engineering, hydrogel, and gene carrier.[20,24–27] Wang et al. investigated the mechanism of ring-opening (co)polymerization of cyclic phosphates[28–30] and synthesized polyphosphoesters with various architectures.[31–36] Iwasaki et al. studied the thermoresponsive behaviors of polyphosphoesters.[37] To be noticeable, the pentavalency of phosphorus allows the chemical linkage of different kinds of side groups to the polymer containing phosphoester,[21] which renders it accessible to prepare molecular brushes. However, the graft polymer based on the polymerization of phosphoesters has been scarcely synthesized except Wang et al.[31]

Amphiphilic copolymers have attracted much attention for their ability to form stable nanosized supermolecular assemblies in aqueous solution.[38] In recent years, the self-assembly behaviors of amphiphilic graft polymers have been extensively investigated for the specific morphologies of the assemblies and their application in biomedical research. Zhang et al. synthesized a series of amphiphilic brushes of poly(*N*-isopropylacrylamide) grafted polyphosphazene (PNIPAm-PPP) and studied the micellization behaviors of these copolymers, which were potential in the application of drug delivery and tissue engineering.[39,40] Gao et al. synthesized branched copolymers with "jellyfish-like" structure, which could form various aggregates with different morphologies, including necklace-like, flower-like onion vesicle, and fiber-like.[7] Zhang[9] and Feng[10] both reported the formation of vesicle structures of the amphiphilic graft copolymers. Other morphologies such as spherical[12,31,41] and *Janus*-type[42] micelles have also been reported. Typical hydrophobic components of these polymers include poly(ε-caprolactone) (PCL)[7,12,31] and polystyrene.[41] Poly(ethylene glycol) (PEG) is one of the most widely used hydrophilic components due to its highly hydrophilic and biocompatible properties.[11,31,32] Several groups have reported the synthesis and micellization of amphiphilic PCL-*graft*-PEG previously,[43–46] while the heterograft structure has rarely been investigated.

In the present work, we report the synthesis of heterograft copolymers via the ring-opening copolymerization of ε-caprolactone (CL) with cyclic phosphoester functionalized PEG (phosPEG) and PCL (phosPCL) macromonomers as demonstrated in Scheme 1. The ratio of hydrophobic and hydrophilic part could be adjusted by the grafting density. The structures and thermal properties of these copolymers were characterized by ^{1}H-NMR, ^{31}P NMR, size exclusion chromatography (SEC), and differential scanning calorimetry (DSC). Their micellar behaviors in aqueous solution were investigated by fluorescence probe technique, dynamic light scattering (DLS), and transmission electron microscopy (TEM).

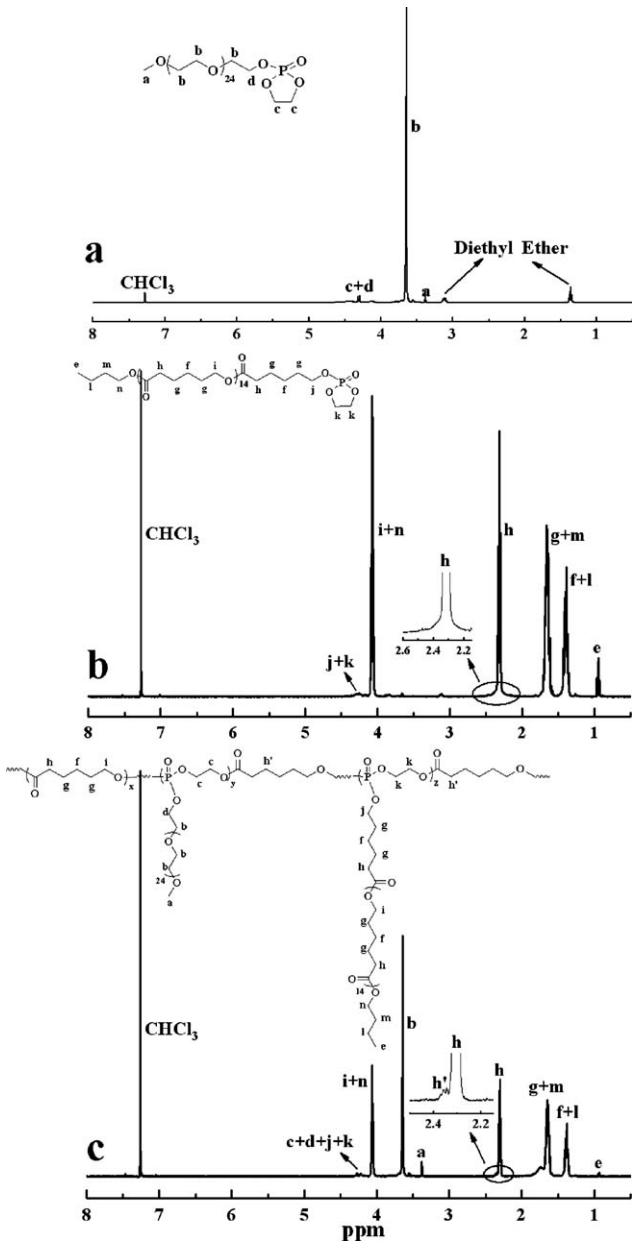


Figure 1 ^{1}H-NMR spectra of (a) phosPEG, (b) phosPCL, and (c) the heterograft copolymer (poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL) as presented in Table I).

EXPERIMENTAL

Materials

2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was synthesized by the method described in literature,[21] (yield: 63%, 82–84°C/50 Pa). Methoxy poly(ethylene glycol) (Fluka product; M_n = 1100 g/mol) denoted as mPEG1100 was dried by azeotropic distillation in the presence of toluene. ε-Caprolactone (Acros product), tetrahydrofuran (THF), triethylamine (TEA), and *n*-butanol were dried over CaH_2 and distilled before use. Stannous octoate [$Sn(Oct)_2$; 97%] and other reagents were purchased from Shanghai Chemical Reagent Co. and used as received.

Experimental

Synthesis of phosphoester functionalized PEG (phosPEG)

Dry mPEG1100 (5.50 g, 5 mmol) and 1 equiv of triethylamine (0.70 mL, 5 mmol) were dissolved in 50 mL of anhydrous THF and cooled to 0°C; 18 mL THF solution of COP (0.46 mL, 5 mmol) was added dropwise under magnetic stirring over a period of 0.5 h. The mixture was further stirred at 0°C for 16 h. The precipitate was filtered off using a Schlenk funnel under argon atmosphere. The filtrate was concentrated under reduced pressure and precipitated into excess cooled anhydrous diethyl ether. The precipitate was collected over the Schlenk funnel and washed by anhydrous diethyl ether three times, and then dried under vacuum to constant weight (yield: 87%).

^{1}H-NMR ($CDCl_3$, ppm): 4.25–4.35 [m, —POC***H***$_2$CH$_2$O(CH$_2$CH$_2$O)$_{24}$—, —OC***H***$_2$CH$_2$OP—], 3.65 [m, —OCH$_2$C***H***$_2$O(C***H***$_2$CH$_2$O)$_{24}$—], 3.38 [s, —OC***H***$_3$] [Fig. 1 (a)].

Synthesis of phosphoester functionalized PCL (phosPCL)

The PCL precursor was polymerized using $Sn(Oct)_2$ as the catalyst and *n*-butanol as the initiator. The polymerization was carried out in a previously flamed and argon-purged 20-mL ampoule. Dry *n*-butanol (0.222 g, 3 mmol) and 15 equiv of CL (5.130 g, 45 mmol) were introduced into the ampoule by a syringe. After stirring for 10 min, $Sn(Oct)_2$ (22.7 mg, 0.056 mmol) was injected into the solution and stirred for additional 10 min. The ampoule was then put into an oil bath at 120°C, 12 h for bulk polymerization. The crude polymer was dissolved in THF and poured into excess cool methanol to precipitate the product, which was dried under vacuum to constant weight (yield: 97%).

phosPCL was synthesized via the reaction of the PCL precursor and COP following the procedures as described for phosPEG (yield: 83%).

^{1}H-NMR ($CDCl_3$, ppm): 4.25–4.38 [m, —POC***H***$_2$(CH$_2$)$_4$CO(O(CH$_2$)$_5$CO)$_{14}$—, —OC***H***$_2$CH$_2$OP—], 4.07 [t, —COOC***H***$_2$(CH$_2$)$_4$CO—, —COOC***H***$_2$CH$_2$CH$_2$CH$_3$], 2.31 [t, —COOCH$_2$(CH$_2$)$_3$C***H***$_2$CO—], 1.65 [m, —COOCH$_2$C***H***$_2$CH$_2$CH$_2$CH$_2$CO—, —COOCH$_2$C***H***$_2$CH$_2$CH$_3$], 1.39 [m, —COOCH$_2$CH$_2$C***H***$_2$CH$_2$CH$_2$CO—, —COOCH$_2$CH$_2$C***H***$_2$CH$_3$], 0.94 [t, —COOCH$_2$CH$_2$CH$_2$C***H***$_3$] [Fig. 1(b)].

Synthesis of heterograft copolymers

Heterograft copolymers were synthesized by the ring-opening copolymerization of CL, phosPEG, and

phosPCL. A typical polymerization procedure was described as follows: phosPEG (1.230 g, 0.99 mmol) and phosPCL (1.177 g, 0.66 mmol) were transferred into a fresh flamed and argon-purged 20-mL ampoule with a magnetic stirring bar. Then CL (1.881 g, 16.5 mmol) was injected into the ampoule. After the two macromonomers were completely dissolved in CL, the ampoule was then connected to a schlenk-line, where an argon exhausting-refilling process was repeated for three times. $Sn(Oct)_2$ (18.4 mg, 0.045 mmol) in 1 mL of dry toluene was injected into the mixture by a syringe, and the exhausting-refilling process was carried out again to remove the toluene. The ampoule was then put into an oil bath at 120°C and the polymerization was performed for 24 h. The crude product was dissolved in THF and dialyzed against distilled water over 7 days (MWCO of dialysis membrane: 14,000). The precipitation was then filtered off through a 0.45-μm filter, and the filtrate was freeze-dried to obtain the final product (yield: 72%).

Preparation of micelles

Micelles were prepared by dialysis technique. Briefly, heterograft copolymer (25 mg) was dissolved in 5 mL THF, then 5 mL distilled water was added dropwise to the solution with vigorous stirring. A light blue tint appeared, which indicates the formation of aggregates. The micelle solution was stirred overnight and dialyzed against distilled water over 2 days to remove THF. The final volume of the aqueous solution was adjusted to 25 mL with the concentration of 1 mg/mL.

Measurements

^1H and ^{31}P NMR spectra were recorded on a Bruker-Avance DMX500 spectrometer at room temperature with $CDCl_3$ as solvent and tetramethylsilane as internal reference. Phosphoric acid (85%) was used as an external reference for ^{31}P NMR measurements.

The molecular weights and molecular weight distributions of these copolymers were determined by size-exclusion chromatography (SEC), which is consisted of a Waters degasser, a Waters-515 HPLC pump with 717 plus autosampler, Waters 2414 RI detector, and columns of Styragel HR 3 and HR 4. The calibration was performed with commercial polystyrene standards. THF was used as the mobile phase with the flow rate of 0.5 mL/min at 35°C.

Differential scanning calorimetry (DSC) measurements were performed on a TA Q100 apparatus. The samples were heated from 0 to 100°C, held for 2 min to erase the thermal history, then cooled to 0°C, and heated again to 100°C at a rate of 10°C/min.

The critical micellization concentration (CMC) was determined by fluorescence measurement using pyrene as fluorescent probe. Fluorescence excitation spectra were recorded on a HITACHI F-4500 fluorescence spectrometer at 390 nm emission wavelength and 2.5 nm slit width. Sample solutions for fluorescence investigation were obtained according to the literature,[47,48] and the concentration of the aqueous solutions ranged from 1.0×10^{-7} to 0.5 mg/mL. The pyrene concentration in the micellar solution was 6.0×10^{-7} mol/L.

The hydrodynamic diameter (D_h) and size distribution of the micelles were determined by dynamic light scattering (DLS) at 90° angle to the incident beam at 25°C on a Brookhaven 90 Plus particle size analyzer. All micellar solutions had a final polymer concentration of 1 mg/mL and were filtered through 0.45 μm filters.

Transmission electron microscopy (TEM) observation was performed on a JEOL JEM-1230 electron microscope operated at an acceleration voltage of 60 kV. The samples were deposited onto the surface of 300 mesh formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. The image contrast was enhanced by negative staining with phosphotungstic acid (2 wt %).

RESULTS AND DISCUSSION

Synthesis and characterization of phosphoester functionalized macromonomers

In this work, PCL was used as the hydrophobic graft section of the copolymer, and PEG as the hydrophilic graft, owing to their attractive biocompatibility and nontoxicity. phosPEG and phosPCL have been synthesized according to the procedures shown in Scheme 1. As presented in our previous work, the PCL precursor was synthesized using *n*-butanol as the initiator in the presence of $Sn(Oct)_2$,[49] which has been the most often used catalyst for the ROP of cyclic lactones for its high activity and U.S. FDA approval as a food additive.[50] PCL with average polymerization degree (DP) of 15 and reasonably narrow molecular weight distribution was synthesized. The DP of PCL was calculated from its ^1H-NMR data based on the integration ratio of methylene protons of CL units (2H, 2.31 ppm, t) and methyl protons of *n*-butanol (3H, 0.94 ppm, t), and thus the average molecular weight of this hydrophobic precursor could be obtained ($M_{NMR} = 1710$).

Both PCL and PEG precursors reacted with COP in the presence of TEA to form the macromonomers with polymerizable cyclic phosphoester ends for

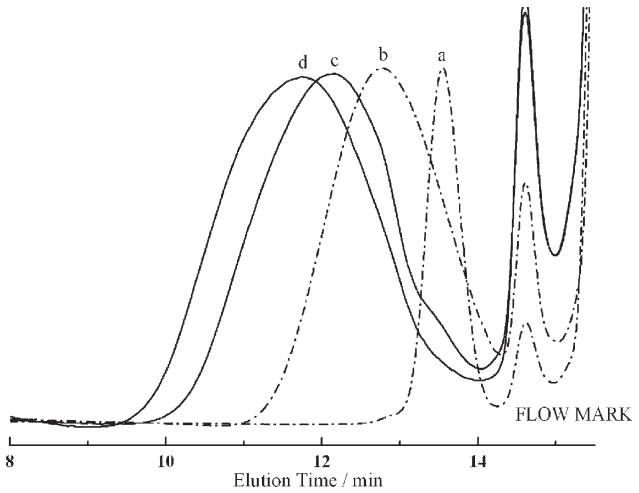


Figure 2 SEC chromatograms of the macromonomers (a) phosPEG and (b) phosPCL; the heterograft copolymers (c) poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL) and (d) poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL).

further ring-opening polymerization. The structures of these macromonomers were confirmed by ^{1}H-NMR spectra shown in Figure 1(a,b), the signals at 4.25–4.40 ppm (c, d, j, and k) are the characteristic signals of methylene protons (—POC***H***$_2$CH$_2$O—) of the cyclic phosphoester and the joint methylene protons (—POC***H***$_2$CH$_2$(OCH$_2$CH$_2$)$_{24}$—, —POC***H***$_2$(CH$_2$)$_4$CO(O(CH$_2$)$_5$CO)$_{14}$—) of the macromonomers. The SEC curves of these two macromonomers are presented in Figure 2, and their molecular weights (M_n) and molecular weight distributions (PDI) are listed in Table I. The molecular weights of phosPEG and phosPCL determined by SEC are higher than those calculated by ^{1}H-NMR data [M_{NMR}(phosPEG) = 1240, M_{NMR}(phosPCL) = 1780], which is ascribed to the fact that monodispersed polystyrene standards were used to generate the calibration curve in SEC analysis.

Synthesis and characterization of heterograft copolymers

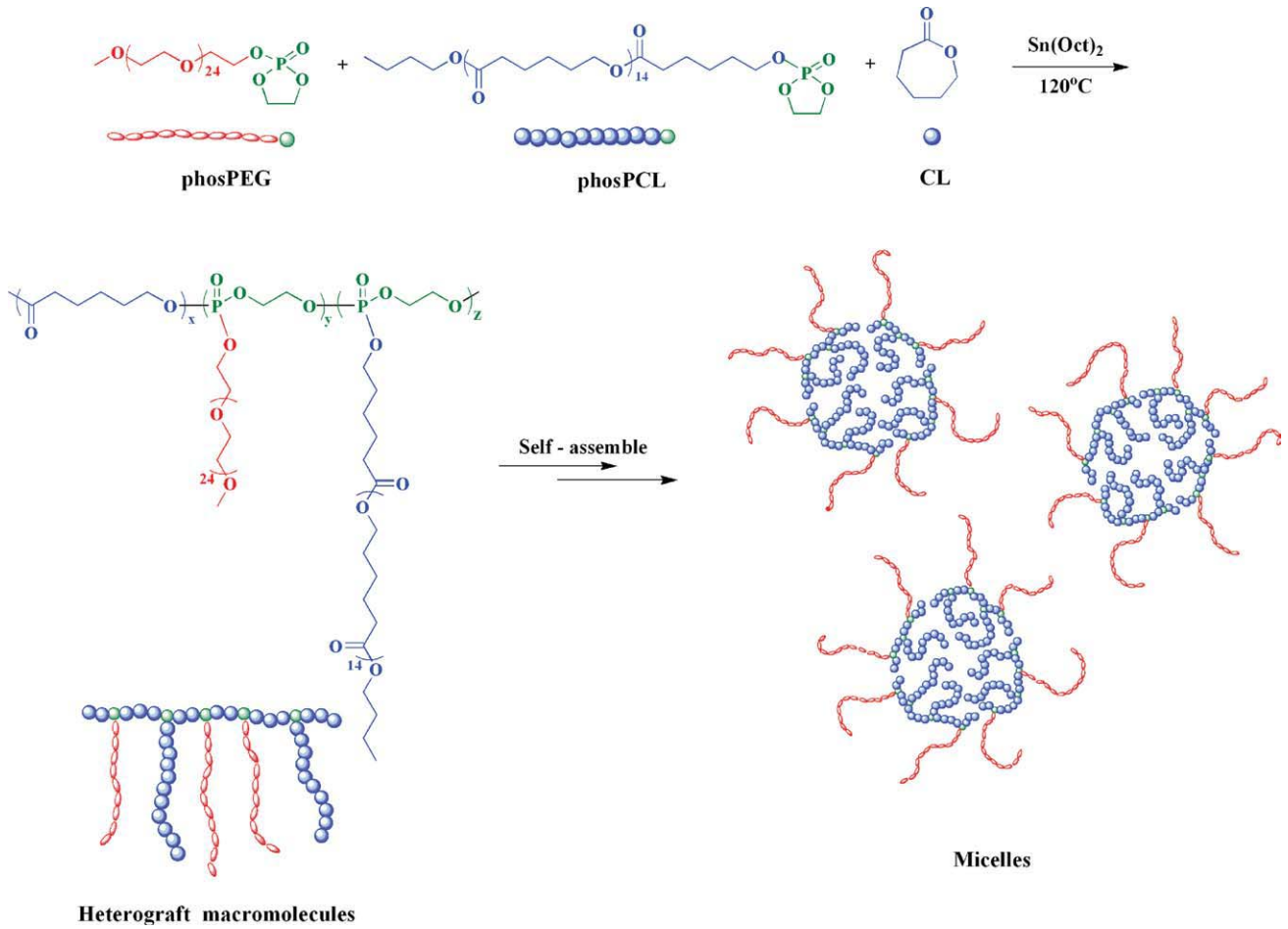
The heterograft copolymers have been synthesized by the ring-opening polymerization of the two phosphoester functionalized macromonomers (phosPEG and phosPCL) and CL in the presence of Sn(Oct)$_2$ as shown in Scheme 2. CL was employed as a comonomer in this reaction to lower the steric hindrance of the grafting. To eliminate the possible unreacted phosPEG and phosPCL, the crude product was further purified by dialyzed in THF/water for 7 days. The PEG macromonomer could be removed by the dialysis of water, and the PCL macromonomer or homopolymer would be precipitated and filtered off thereafter. The heterograft copolymer was finally obtained after freeze-drying. To show the effect of purification, similar procedures were taken for the mixture of phosPEG, phosPCL, and PCL, and there was no product left after dialysis and freeze-drying.

Heterograft copolymers with various compositions were synthesized and listed in Table I. A typical ^{1}H-NMR spectrum of these copolymers (poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL)) and the assignments of the peaks are presented in Figure 1(c). All the signals of phosPEG and phosPCL shown in Figure 1(a,b) are detected. As both the unreacted monomers have been eliminated over the process of dialysis, these signals suggest that the two macromonomers should be "grafting through" the backbone. The intensity ratio of H^h/H^c is much higher than that in Figure 1(b), which demonstrates the incorporation of CL units in the copolymer. Notably, comparing with Figure 1(b), there is an additional weak peak ($H^{h'}$) at around 2.35 ppm, which is assigned to the characteristic signal of the last —C***H***$_2$— group in CL segments next to the phosphoester units. This signal confirms the phosphoester functionalized macromonomers randomly embed in the CL segments in the backbone, as shown in Scheme 2.

TABLE I
Synthesis and Characterization of Heterograft Copolymers via "Grafting Through" by Ring-Opening Copolymeization[a]

Entry	Feed ratio (phosPEG: phosPCL:CL)	DP ratio[b] (phosPEG: phosPCL:CL)	Unit ratio[b] (EO:CL)	M_n[c]	PDI[c]
phosPCL				3.6	1.34
phosPEG				1.7	1.06
Poly(phosPEG-*co*-CL)	10:0:100	6.9:0:100	174.5:100	5.3	1.42
Poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL)[d]	9:1:100	5.9:0.7:100	131.1:100	7.5	1.39
Poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL)	6:4:100	4.4:2.1:100	84.7:100	10.0	1.52
Poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL)	3:7:100	1.9:4.8:100	29.4:100	6.7	1.46

[a] Polymerization conditions: bulk, 120°C, 24 h.
[b] DP ratios of the three monomers and unit ratios of ethylene oxide (EO) and CL in the copolymers, calculated from ^{1}H NMR data by eqs. (1) and (2) discussed later.
[c] Measured by SEC with monodispersed polystyrene standards.
[d] 0.9 represents the molar fraction of phosPEG in all macromonomer, similarly hereinafter.



Scheme 2 Synthesis and preparation of heterograft copolymer micelles via the "grafting through" method [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.].

To determine the grafting efficiency of the macromonomers and the composition of the heterograft copolymers, two parameters, DP ratios of the three monomers and unit ratios of ethylene oxide (EO) and CL, were introduced and summarized in Table I. They can be calculated from ^{1}H-NMR data by the following eq. (1) and (2):

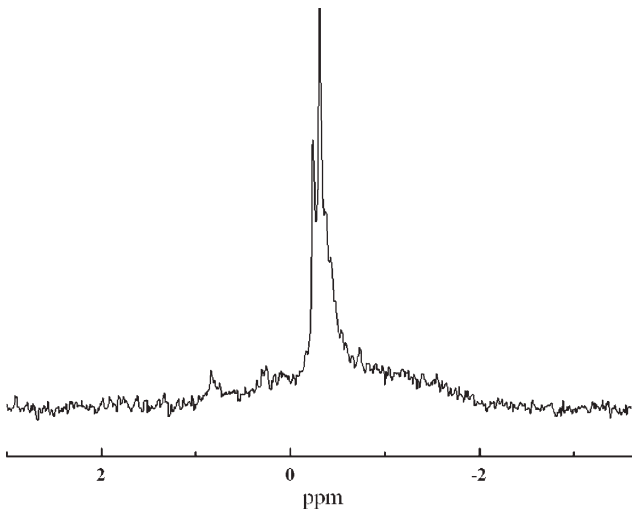


Figure 3 ^{31}P NMR spectrum of the heterograft copolymer (poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL) as presented in Table I).

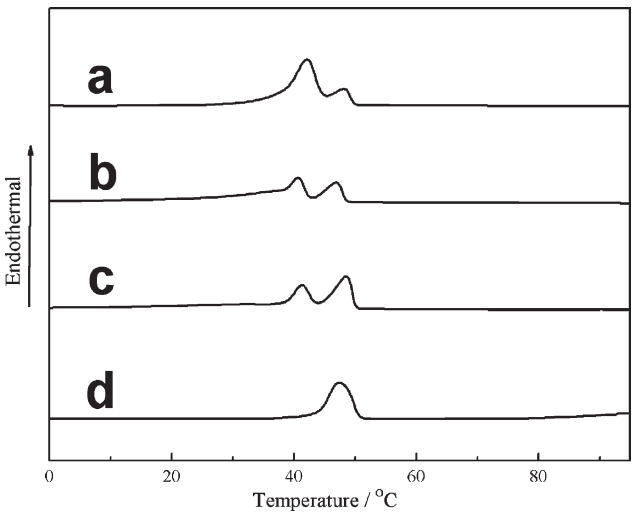


Figure 4 The DSC curves (second heating run) of the graft copolymers: (a) poly(phosPEG-*co*-CL); (b) poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL); (c) poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL); and (d) poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL).

TABLE II
Thermal and Micellar Properties of the Heterograft Copolymers

Entry	T_m (°C)[a]		ΔH_m (J/g)[a]		D_h[b] (nm)	PDI[b]	CMC[c] (mg/L)
	PEG	PCL	PEG	PCL			
Poly(phosPEG-*co*-CL)	42.1	48.4	31.4	6.8	20.5	0.268	1.25
Poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL)	41.1	47.6	14.1	12.8	35.4	0.346	1.09
Poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL)	41.5	48.5	11.4	20.9	41.5	0.327	0.95
Poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL)	–	48.3	–	46.7	53.5	0.283	0.69

[a] The melting temperature (T_m) and fusion enthalpy (ΔH_m) of the heterograft copolymers, determined by DSC measurements.
[b] The hydrodynamic diameter (D_h) and size distribution (PDI) of the heterograft copolymer micelles, determined by DLS measurements.
[c] The critical micelle concentration (CMC)s of the heterograft copolymers, determined by fluorescence technique using pyrene as a probe.

$$\text{DP ratio (phosPEG:phosPCL:CL)} = \frac{I_{H^a}}{3} : \frac{I_{H^e}}{3} : \frac{I_{H^h} - 10I_{H^e}}{2} \quad (1)$$

$$\text{Unit ratio (EO : CL)} = \frac{I_{H^b}}{4} : \frac{I_{H^h}}{2} \quad (2)$$

in which, I_{H^a}, I_{H^b}, I_{H^e}, and I_{H^h} represent the integration of H^a, H^b, H^e, and H^h in Figure 1(c), respectively. Comparing with the feed ratio, the macromonomers showed lower polymerization activities than CL, which is originated from the low concentration of the polymerizable phosphoester groups and high steric hindrance of the macromonomers.[51]

Figure 3 presents a typical ^{31}P NMR spectrum of these heterograft copolymers (poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL)). Two small peaks at around −0.33 ppm have been detected, which are assigned to the two kinds of phosphorus [—*P*OCH$_2$(CH$_2$)$_4$CO (O(CH$_2$)$_5$ CO)$_{14}$—, —*P*OCH$_2$CH$_2$O(CH$_2$CH$_2$O)$_{24}$—] originated from the two macromonomers in the copolymers. As

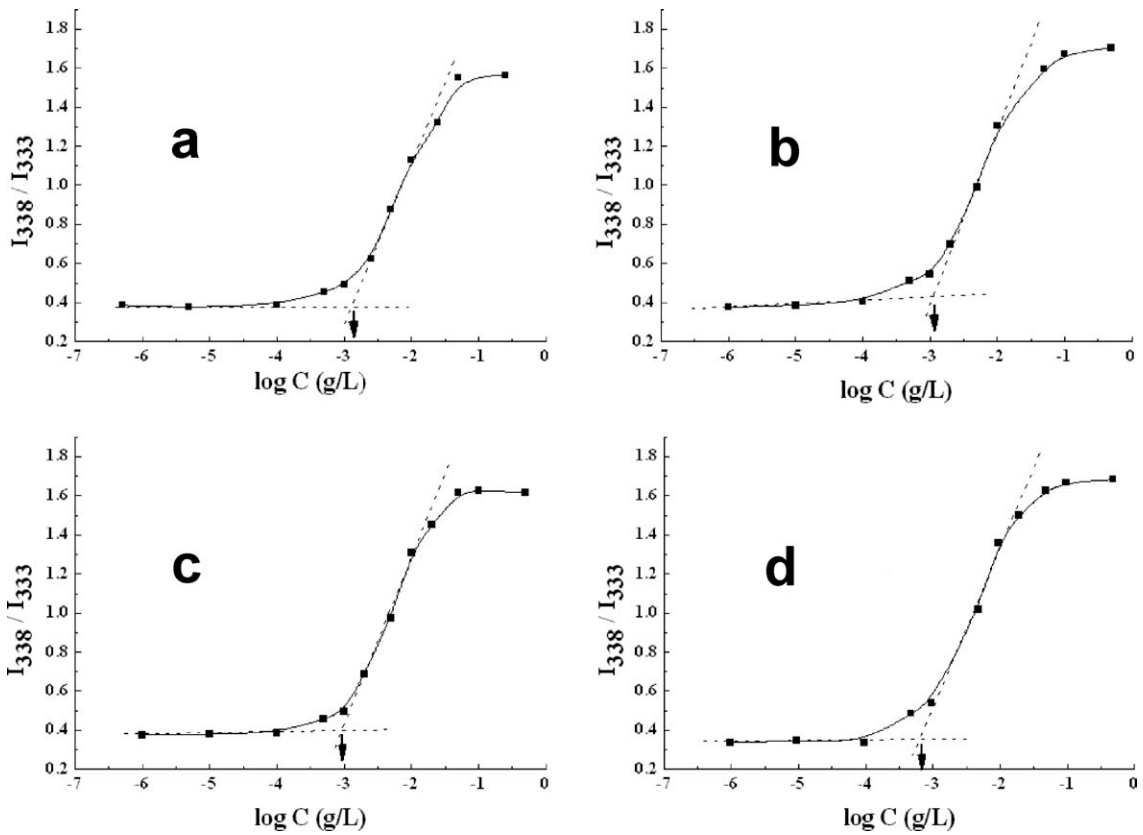


Figure 5 Plots of the I_{338}/I_{333} ratio (from pyrene excitation spectra) versus log C for the heterograft copolymers: (a) poly(phosPEG-*co*-CL); (b) poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL); (c) poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL); and (d) poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL).

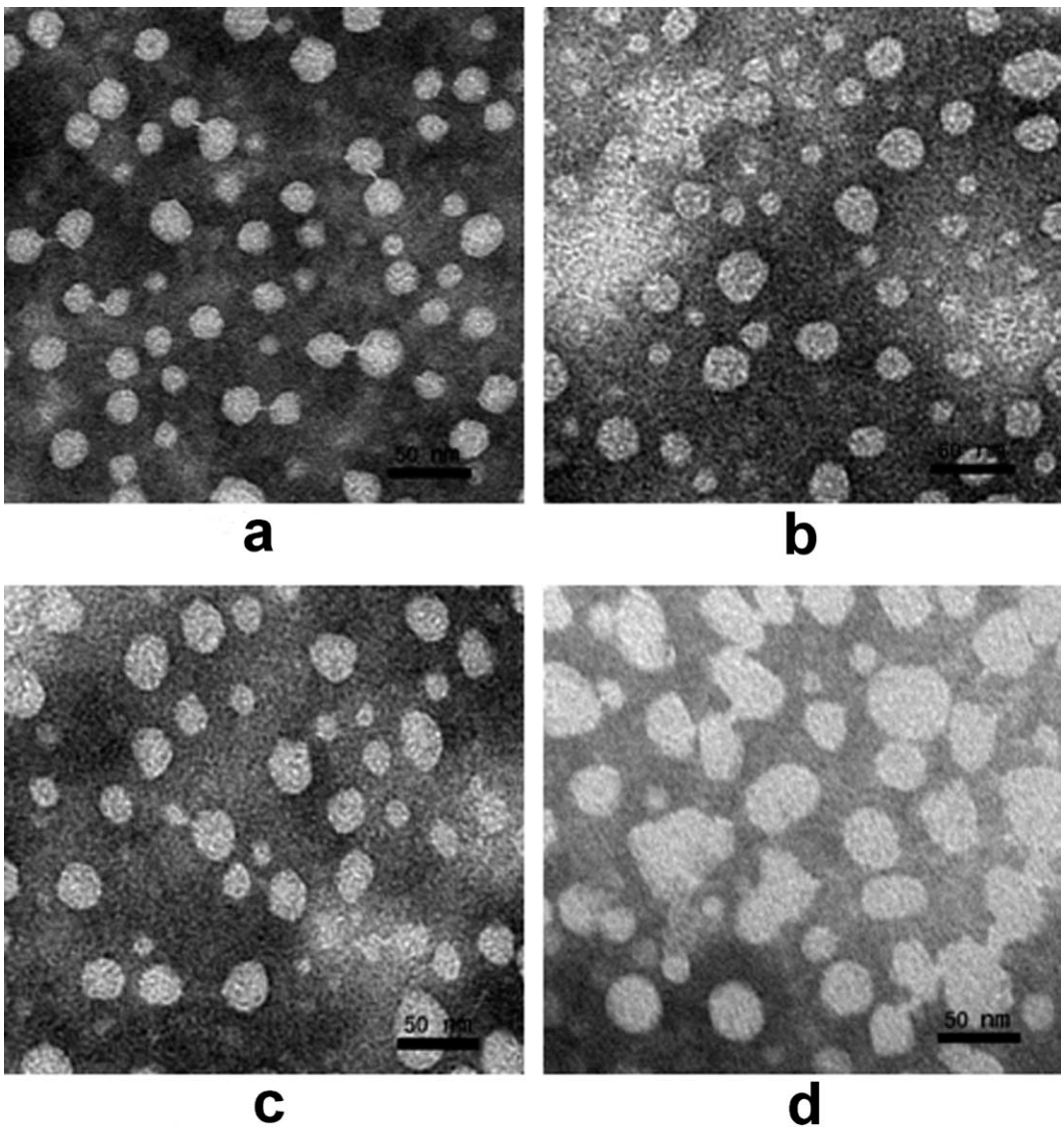


Figure 6 TEM micrographs of the heterograft copolymers: (a) poly(phosPEG-*co*-CL); (b) poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL); (c) poly(phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL); and (d) poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL).

both the phosPEG and phosPCL have been removed in the process of dialysis, these signals reconfirm that both the two functionalized macromonomers have been successfully incorporated in the backbones, which demonstrates the heterograft structure of the copolymers.

The SEC curves of the typical heterograft copolymers are shown in Figure 2. Each chromatograph shows a unimodal peak, which suggests there was no homopolymers or macromonomers left in the final products. The average molecular weights (M_n) and molecular weight distributions (PDI) of the copolymers are listed in Table I. Comparing with the two macromonomers, the molecular weights of the copolymers increase but not apparently as expected, especially for poly(phosPEG-*co*-CL). That might be attributed to two factors: first, the low concentration of polymerizable phosphoester groups and the high steric hindrance of the macromonomers rendered the "grafting through" process difficult, as discussed above; second, the graft copolymer with highly branched structure would have relatively smaller hydrodynamic volume than that of linear macromolecule.[3,52]

The thermal properties of these heterograft copolymers were analyzed by DSC with the curves of second heating run presented in Figure 4. The melting temperatures and fusion enthalpies of the copolymers are listed in Table II. Two endothermic peaks have been detected for poly(phosPEG-*co*-CL), poly(phosPEG$_{0.9}$-*co*-phosPCL$_{0.1}$-*co*-CL), and poly (phosPEG$_{0.6}$-*co*-phosPCL$_{0.4}$-*co*-CL), which refer to the melting transitions of PEG and PCL, respectively. With the lowering of unit ratio (EO: CL), the

enthalpy of PEG decreases and that of PCL increases. The melting transition of PEG was not detected for poly(phosPEG$_{0.3}$-*co*-phosPCL$_{0.7}$-*co*-CL), resulting because too small amount of PEG in this copolymer could not crystallize with the influence of large amounts of PCL.[53]

Micellization of heterograft copolymers

Amphiphilic graft copolymers could self-assemble to form micelles with specific structures in aqueous media as discussed above. However, the micellar behaviors of heterograft copolymers have been rarely studied except that Ishizu et al. have synthesized amphiphilic heterograft brushes with PEG and PHEMA side chains, which demonstrated micelles similar to *Janus*-type.[42] To study the micellar behaviors of the heterograft copolymers herein with different compositions of PCL and PEG, the fluorescence technique has been employed and pyrene was used as a probe. As reported by Wilhelm et al.,[47,48] pyrene molecules will transfer into hydrophobic microdomains with a concurrent change in the molecule's photophysical properties. In the excitation spectra, a sharp rise in the intensity ratio of the peaks at 338 and 333 nm of pyrene indicates the onset of micellization (CMC) for the amphiphilic copolymers.

Figure 5 plots the ratio of intensities (I_{338}/I_{333}) versus logarithmic concentration (log C) of the heterograft copolymers. At lower concentrations, this ratio takes the characteristic value of pyrene in water; and at higher concentrations (above CMC), it takes the value of pyrene entirely in the hydrophobic environment afforded by the micellar core. The CMC was determined from the crossover point at the low concentration range, which is demonstrated by the dashed line in Figure 5. As shown in Table II, the CMC values of these heterograft copolymers are in the range of 0.69–1.25 mg/L, increasing as the content of hydrophobic PCL decreases. It is reasonable that the higher content of the hydrophobic segments will lead to stronger interactions between each other in the aqueous solution, resulting in a more stable micellar structure and lower CMC value, as reported by Wang et al.[31,36]

The formation of micelles was further confirmed by TEM measurement as shown in Figure 6. It can be observed that all the micelles take a spherical morphology. The particle sizes of these micelles were measured by the mean of DLS with the results presented in Table II. The hydrodynamic diameters (D_h) of these micelles are in the range of 20–60 nm, increasing as the content of PCL increases, which resulted mainly from the enhanced hydrophobic property by more PCL chains in the copolymer. It is noticeable that the size values estimated by TEM from Figure 6 are similar to those obtained from the DLS measurements (Table II), and the size-changing trend in TEM images is in good agreement with the DLS data.

CONCLUSIONS

Novel heterograft copolymers have been synthesized by the ring-opening copolymerization of CL and macromonomers functionalized by cyclic-phosphoester via the method of "grafting through." The structure and thermal properties of these brushed copolymers were characterized. These heterograft copolymers are amphiphilic and could form spherical micelles in aqueous solution, which demonstrates their potential in biomedical application.

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