# Synthesis and Characterization of Amphiphilic Polyphosphates with Hydrophilic Graft Chains and Cholesteryl Groups as Nanocarriers

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Amphiphilic polyphosphate graft copolymers with varied densities of cholesteryl esters and hydrophilic graft chains were prepared, and the solution properties of the graft copolymers were evaluated. Polyphosphates were synthesized as backbones by ring-opening polymerization of 2-isopropyl-2-oxo-1,3,2-dioxaphospholane (IPP), 2-(2-oxo-1,3,2-dioxaphosphoroyloxyethyl-2-bromoisobutyrate) (OPBB), and 2-choresteryl-2-oxo-1,3,2-dioxaphospholane (ChOP) using triisobutylaluminum as an initiator. Three types of polyphosphates ($PIBr_xCh_y$, $x$ = number of OPBB units in a polymer; $y$ = number of ChOP units in a polymer) such as $PIBr_4$, $PIBr_6Ch_1$, and $PIBr_3Ch_2$ were obtained. The molecular weights of these polymers were $2.4 \times 10^4$, $2.4 \times 10^4$, and $2.6 \times 10^4$ g/mol, respectively. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was grafted from the OPBB sites in $PIBr_xCh_y$ via atom transfer radical polymerization (ATRP) in EtOH. In each polymer system, the molecular weight of the graft polymer was linear with conversion. Furthermore, the polymer radical concentration remained constant during polymerization; that is, the molecular weights of the graft chains were easily controllable with polymerization time. The solution properties of amphiphilic $PIBr_xCh_y$-*g*-PMPCs were investigated by the methods of surface tension measurement, light scattering, and fluorescence probe. The transition point (*cmc*) of the surface tension of the $PIBr_xCh_y$-*g*-PMPCs aqueous solution decreased with an increase in the number of ChOP units in a graft polymer. Particularly, $PIBr_3Ch_2$-*g*-PMPC14.9K formed nanosized associates ($R_h$ = 7.5 nm) with 2.2 molecules above 0.1 wt %. v79 cells were used to evaluate the cytotoxicity of the graft polymers, but no cytotoxicity was observed. The graft polymers containing cholesteryl groups effectively enhanced the solubility of paclitaxel in an aqueous solution.

## Introduction

Polymeric amphiphiles have been studied as bio-related materials due to their self-assembled nature.[1] More recent trends in the study of amphiphilic polymers are the solubility enhancement of hydrophobic drugs[2] and injectable thermoresponsive hydrogels for tissue engineering.[3] Well-defined amphiphilic copolymers are interesting because they form varied aqueous solution properties by simple monomer combinations. Atom transfer radical polymerization (ATRP) is one of the best methods for synthesizing well-defined polymers because it can be applied to the polymerization of an extensive variety of monomers.[4−7] ATRP has great synthetic capability in controlling the molecular architecture of polymers and provides an exceptionally robust method of producing block or graft copolymers.[4,8−15] ATRP has been used to synthesize numerous amphiphilic polymers.[5,16−23]

There has been a great deal of interest in polyphosphates, which are biodegradable through hydrolysis and, possibly, through enzymatic digestion of phosphate linkages under physiological conditions.[24] Biodegradable polyphosphates appear attractive for biological and pharmaceutical applications because of their biocompatibility and structural similarities to naturally occurring nucleic and teichoic acids. Recently, polyphosphates have been proposed for use in the field of tissue engineering as scaffolds and as gene carriers.[25−28]

We have synthesized biodegradable amphiphilic polymers consisting of hydrophobic polyphosphates prepared by ring-opening polymerization and the well-defined poly[2-methacryloyloxyethyl phosphorylcholine (MPC)] graft chain.[29] MPC polymers are well-known as biocompatible polymers, which have been synthesized by mimicking biomembrane structures.[30−33] The amphiphilicity of these graft polymers were well controllable by changing the molecular weight and density of the grafting poly(MPC) chain. Nevertheless, the applicability of graft polymers for biomedical applications is still uncertain.

The hydrophobicity of the polyphosphate backbone of graft polymers can be controlled by changing the chemical structure of side chains and may influence polymer association. The cholesteryl group is one of the most probable groups for controlling hydrophobic interaction. We have studied physically cross-linked nanogels by the controlled association of the cholesteryl group-bearing pullulan (CHP).[34,35] The CHP molecules associated in dilute aqueous solution and formed monodispersed nanogels in which the associations of the hydrophobic groups provided physical cross-linking points. An interesting property of the CHP nanogels is their ability to form complexes with a variety of soluble proteins in water.[36] The nanogels can be used as drug-carrier systems in medicine[37] and as artificial molecular chaperones in biotechnology.[38,39]

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**Table 1.** Synthetic Results of Polyphosphates

| polyphosphate | OPBB/IPP/ChOP (mol %) in feed | OPBB/IPP/ChOP (mol %) in copolymer[a] | yield (%) | $M_w$[b] ($\times 10^{-4}$) | $M_w/M_n$[b] | no. of OPBB[a] per a polymer ($x$) | no. of cholesterol[a] per a polymer ($y$) |
|---|---|---|---|---|---|---|---|
| $PIBr_4$ | 4.0/96.0/- | 2.6/97.4/- | 73.2 | 2.6 | 1.3 | 4.0 | |
| $PIBr_6Ch_1$ | 4.5/94.5/1.0 | 4.3/95.1/0.6 | 58.0 | 2.4 | 1.5 | 5.7 | 0.9 |
| $PIBr_3Ch_2$ | 4.0/04.0/2.0 | 2.1/96.8/1.2 | 62.5 | 2.4 | 1.3 | 2.9 | 1.7 |

[a] Determined by $^1$H NMR. [b] Apparent molecular weight: Determined by GPC.

Here, we report new syntheses of amphiphilic graft polymers bearing cholesteryl groups in a polyphosphate backbone. In addition, the in vitro cytotoxicity of the polymers and enhancement of paclitaxel (PTX) solubility with the graft polymers were also investigated.

## Materials and Method

**Materials.** Diethyl ether, 2-propanol, and diisopropylamine (DIPA) were purified by conventional distillation. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) was synthesized according to the method of Edmundson, purified by distillation under reduced pressure, and the fraction of bp 98 °C/1 mmHg (lit.: bp 79°C/0.4 mmHg) was used.[40] 2-Isopropyl-2-oxo-1,3,2-dioxaphospholane (IPP) and 2-(2-oxo,1,3,2-dioxaphospholoyloxy) ethyl-2′-bromoisobutyrate (OPBB) were synthesized as previously reported[29,41] and purified by vacuum distillation and recrystallization from diethyl ether, respectively. They were stored under argon at −30 °C until use. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized by the method previously described and purified by recrystallization from acetonitrile.[42] Cholesterol was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Copper(I) bromide, 2,2′-bipyridine (bpy), and 2-bromoisobutyryl bromide were all purchased from Aldrich and used without further purification. 8-Anilino-1-naphthalene sulfonic acid sodium salt (ANS) was purchased from Tokyo Kasei Co., Ltd., Japan, and used without further purification. Distilled water was obtained by purification using a Millipore Milli-Q system that involves reverse osmosis, ion exchange, and filtration (18.2 MΩ).

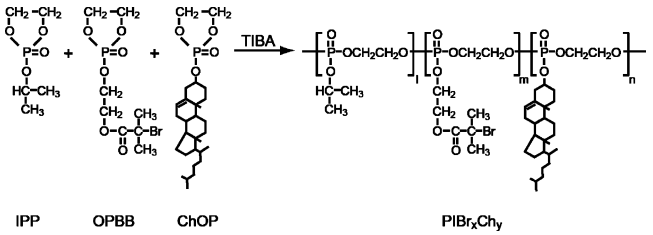
**Synthesis of Cyclic Phosphate Having a Cholesteryl Group.** COP (0.047 mol) was added dropwise to a diethyl ether solution (300 mL) of DIPA (0.047 mol) and cholesterol (0.047 mol) at −30 °C for a period of 1 h. The reaction was allowed to continue at −30 °C for another 2 h. The reaction mixture was filtered to collect the precipitate including diisopropylammonium chloride. The solvent was then reduced by half, and the remaining diethyl ether solution was cooled to 0 °C. 2-Cholesteryl-2-oxo-1,3,2-dioxaphospholane (ChOP) was obtained by recrystallization in 32.5% yield. The structure of ChOP was confirmed by $^1$H NMR (α-500, JEOL, Tokyo, Japan) and FT-IR spectroscopy (FT-500, Jasco, Tokyo, Japan).

$^1$H NMR (500 MHz, $CDCl_3$): $\delta =$ ChOP: 0.65 (s; H of $CH_3$ from cholesterol, 3H), 0.85 (m; H of $(CH_3)_2$ from cholesterol, 6H), 0.95 (m; H of $CH_3$ from cholesterol, 3H), 1.10 (m; H of $CH_3$ from cholesterol, 3H), 0.70−2.50 (m; H from $CH_2$–$CH_2$ and $CHCH_2$ from cholesterol, 17H), 4.35−4.48 (m; $-OCH_2CH_2O-$ in cyclic phosphate, 4H).

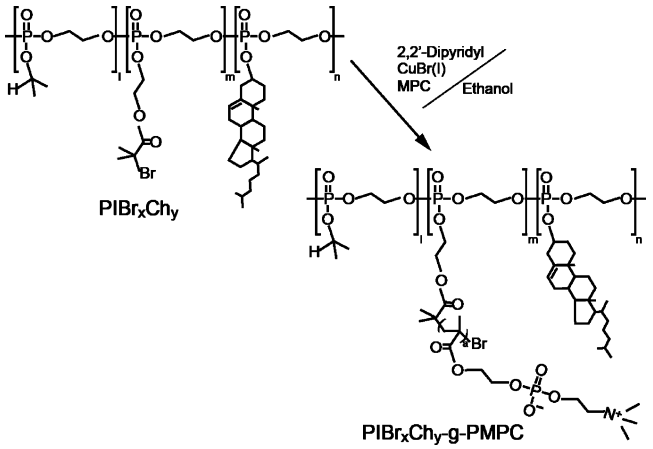
IR: 2910 ($-CH_2-$), 1235 ($-OPO-$), 1089 ($-OPO-CH_2-$) $cm^{-1}$.

**Synthesis of Polyphosphates.** A given amount of IPP, OPBB, and ChOP were placed into a thoroughly dried 50-mL round-bottomed flask equipped with a three-way stopcock. After the mixture was heated at 70 °C and dried under reduced pressure for 2 h, triisobutyl aluminum was added under an argon gas atmosphere. The reaction continued until the magnetic stirring bar stopped due to the increased viscosity of the mixture (approximately 0.5−1 h). Dry THF was then added to dilute the reaction mixture. The polyphosphate ($PIBr_xCh_y$; $x$ and $y$ represent the number of OPBB and ChOP units in a polyphosphate, respectively) was purified by reprecipitation from diethyl ether. Scheme 1 and Table 1 show the chemical structure and the synthetic results of the $PIBr_x$-$Ch_y$, respectively.

**Scheme 1.** Synthetic Route of Polyphosphate




**Scheme 2.** Synthetic Route of Polyphosphate Grafted with Poly(MPC) via ATRP




The weight-averaged molecular weights of the $PIBr_xCh_y$s were measured using gel-permeation chromatography (GPC) through a Shodex KF-803 column using a calibration curve based on linear polystyrene standards. THF was used as solvent for GPC measurement. The mole fraction of each unit of the $PIBr_xCh_y$ was calculated from $^1$H NMR data.

**ATRP of MPC from Polyphosphate.** Graft polymers were prepared by a procedure similar to that reported previously.[29] The synthetic route of a graft polymer is shown in Scheme 2. Briefly, a given amount of $PIBr_xCh_y$ (OPBB unit; 0.067 mmol) was dissolved in ethanol, and argon gas was passed through the solution for 30 min to eliminate any oxygen. Cu(I)Br (9.5 mg, 0.067 mmol) and bpy ligands (21.0 mg, 0.135 mmol) were added to the stirred solution under argon after which MPC (2.00 g, 6.73 mmol) was added to the reaction mixture under argon. The solution was stirred at room temperature for 12 h. After polymerization, the graft copolymer [$PIBr_xCh_y$-*g*-poly(MPC) (PMPC)] was precipitated into THF, then dissolved in water, and passed through a silica gel column to remove any residual ATRP catalyst. In addition, the elution was dialyzed using Spectra/Pro molecular weight cut off regenerated cellulose membranes (MWCO 3500) for 3 days to remove any unpolymerized MPC.

The conversion of $PIBr_xCh_y$-*g*-PMPC was calculated from the $^1$H NMR data. The apparent molecular weight of $PIBr_xCh_y$-*g*-PMPC was determined by GPC with a Tosoh GPC system having a refractive index detector and size-exclusion columns, Shodex, SB-804 HQ with a poly(ethylene glycol) (PEG, Tosoh standard sample) standard in distilled water containing 10 mM LiBr.

**Amphiphilic Properties of $PIBr_xCh_y$-g-PMPC.** The surface tensions of the $PIBr_xCh_y$-*g*-PMPC aqueous solutions at various concentra-

tions were measured by the Wilhelmy method with a dynamic contact angle meter (DCA-100, Orientec, Co., Ltd., Tokyo, Japan).

The absolute molecular weight ($M_w$) of each of the $PIBr_xCh_y$-*g*-PMPCs was measured by multi-angle laser light scattering (MALLS) analysis in batch mode (Wyatt Dawn DSP−F detector) above the critical micelle concentration (*cmc*). The refractive index increment (d*n*/d*c*) for the $PIBr_xCh_y$-*g*-PMPC was measured using a Wyatt Optilab DSP detector with a 690-nm light. The absolute $M_w$ was determined by Zimm extrapolation to zero angle and zero concentration for a series of measurements for five solutions at angles ranging from 40° to 130°.

The absolute $M_w$, the mean-squared radius of gyration ($R_g$), and the second virial coefficient ($A_2$) for graft copolymer associates were determined from MALLS analysis using the following equation:

$$\frac{K_c}{R_\theta} = \frac{1}{M_w}\left(1 + \frac{16\pi^2}{3\lambda^2}R_g^2 \sin^2\frac{\theta}{2}\right) + 2A_2c \quad (1)$$

where $c$ denotes the weight concentration of the polymer, $R_\theta$ is the Rayleigh ratio, $\theta$ is the scattering angle, $\lambda$ is the wavelength of light in the medium, and $K$ is given as

$$K = (4\pi^2/\lambda_0^4 N_A)n_0^2(dn/dc)^2 \quad (2)$$

where $\lambda_0$ is the wavelength of light in a vacuum, $N_A$ is Avogadro's number, $n_0$ is the refractive index of the medium, and d*n*/d*c* is the change in the refractive index with the concentration of polymers.

The interaction of ANS with a polymer associate was studied to estimate the polarity of the hydrophobic domain inside the polymeric associate. A fixed amount of $PIBr_xCh_y$-*g*-PMPC was dissolved in $1.0 \times 10^{-5}$ M ANS aqueous solution. The $PIBr_xCh_y$-*g*-PMPC solution was further diluted with ANS aqueous solution ($1.0 \times 10^{-5}$) to a desired concentration of polymer. The internal polarity of the polymer aggregates was evaluated by the maximum wavelength from the fluorescence spectra of ANS ($\lambda_{ex}$ = 350 nm, measurement range of 420 nm − 620 nm).

The size of the polymeric associate of $PIBr_3Ch_2$-*g*-PMPC was determined by dynamic light scattering (Zetasizer Nano ZS; Malvern Instruments, Ltd., Worcestershire, U.K.). The scattering was performed with a vertically polarized incident beam at a wavelength of 633 nm supplied by a He−Ne ion laser. The measurements were performed with a scattering angle of 90°, the measurement temperature was 25 °C, and the concentration of $PIBr_xCh_y$-*g*-PMPC was 0.5 g/dL.

**Cytotoxity Test of $PIBr_xCh_y$-*g*-PMPC.** Chinese hamster fibroblasts (v79 cells) were purchased from RIKEN Cell Bank. The v79 cells were maintained in a culture medium (Eagle's MEM; Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum at 37 °C in a humidified atmosphere of air containing 5% $CO_2$. The contents of the flasks used for cell preservation were detached by trypsin treatment and 50 cells in 1 mL culture medium were seeded in each of 24 wells. The cells were stored overnight at 37 °C in a $CO_2$ incubator with 95% humidity to adhere to the well surfaces. A specific amount of graft polymers was then introduced into the culture media. Zinc diethyldithiocarbamate (ZDEC) and zinc dibutyldithiocarbamate (ZDBC) were used as probe cytotoxic compounds.[43] The cells were cultured for 7 days in a $CO_2$ incubator. To fix the colony that formed on the surface, the wells were rinsed with PBS and treated with 10% formaldehyde solution. After being washed with water, the colony was stained with 10% Giemsa solution, and the number of colonies was counted.

**Determination of PTX Solubility in Graft Polymer Aqueous Solutions.** PTX (1.0−5.0 mg) was dissolved in 100 $\mu$L of ethanol. The PTX solution was then added to 900 $\mu$L of an aqueous solution containing 10 mg/mL of graft polymer (PTX concentration, 0.1−0.5 mg/mL; graft polymer concentration, 9 mg/mL), followed by vortexing of the mixture solution. The ethanol was removed from the solution under reduced pressure. A photograph of the aqueous solution was then taken.

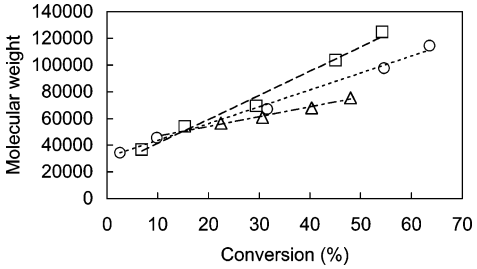



**Figure 1.** Dependence of Mn on conversion in the polymerization of MPC initiated from polyphosphates. ○, $PIBr_4$-*g*-PMPC; □, $PIBr_6Ch_1$-*g*-PMPC; △, $PIBr_3Ch_2$-*g*-PMPC.

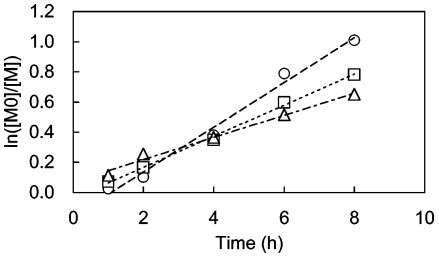



**Figure 2.** Kinetics of ATRP of MPC initiated from polyphosphates. ○, $PIBr_4$-*g*-PMPC; □, $PIBr_6Ch_1$-*g*-PMPC; △, $PIBr_3Ch_2$-*g*-PMPC.

## Results and Discussion

**Synthesis of Polyphosphates.** The chemical structure and synthetic results of the polyphosphates are shown in Scheme 1 and Table 1, respectively. Polymerization was homogeneously performed by a solvent-free reaction. To dissolve ChOP in a monomer solution, the solution was heated to 70 °C and kept at that temperature during polymerization. As indicated in Table 1, the composition of ChOP in the copolymer was relatively lower than that in the feed. The polyphosphates synthesized in this study were soluble in ethanol, THF, and chloroform but not in water and diethyl ether. The polymer was stored in dry ethanol solution at −80 °C until use. Three kinds of polyphosphates were synthesized in this study. $PIBr_4$, $PIBr_6Ch_1$, and $PIBr_3Ch_2$ contain 4.0, 5.7, and 2.9 initiator sites, and 0, 0.9, and 1.7 cholesteryl groups per molecule, respectively. The molecular weight of the polyphosphates was $2.4 \times 10^4$−$2.6 \times 10^4$.

**Synthesis of Graft Copolymers via ATRP.** ATRP of the MPC from polyphosphate was performed in an ethanol solution. Figure 1 shows the relationship between the molecular weight determined by GPC and the conversion of the MPC polymers grafted from polyphosphates. In every polymer system, the molecular weight of the graft polymer is linear with conversion. Figure 2 shows that the monomer consumption followed first-order kinetics. The semilogarithmic plot indicates that polymerization is first order with respect to MPC and implies that the polymer radical concentration remains constant on the polymerization time scale. In previous literature, we reported that graft polymers were rapidly degraded in basic buffer solutions due to the hydrolysis of the polyphosphates main chain and that homogeneous poly(MPC) was obtained after degradation.[29] In $PIBr_xCh_y$, the polymerization may proceed homogeneously at every initiator site. Following this study, graft polymers synthesized by 6-h polymerization were used. The numbers behind the PMPC of $PIBr_xCh_y$-*g*-PMPC correspond to the absolute molecular weight of the PMPC graft chain, which was calculated from the $^1$H NMR ratio of a trimethylammonium group of PMPC to the isopropyl group of $PIBr_xCh_y$. Schematic representation of selected graft polymers and their characterizations are shown in Figure 3 and Table 2, respectively.

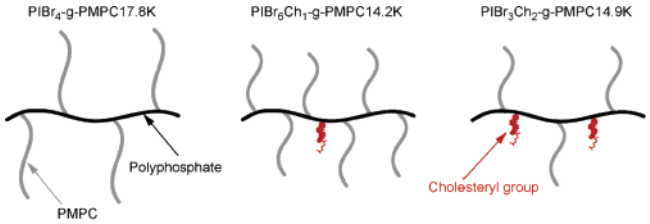



**Figure 3.** Schematic representation of graft copolymers for evaluation of solution properties

**Table 2.** Molecular Weight of Selected Graft Copolymers

| graft polymer | molecular weight (x10$^4$) | |
|---|---|---|
| | $M_w$ from $^1$H NMR[a] | $M_w$ from GPC |
| PIBr$_4$-*g*-PMPC17.8K | 9.7 | 11.8 |
| PIBr$_6$Chol$_1$-*g*-PMPC14.2K | 10.5 | 8.2 |
| PIBr$_3$Chol$_2$-*g*-PMPC14.9K | 6.7 | 5.1 |

[a] The molecular weight of the polyphosphate was determined GPC because the molecular weight was similar to that determined with MALLS.[29] Ethanol-$d_6$ was used as solvent for $^1$H NMR analysis.

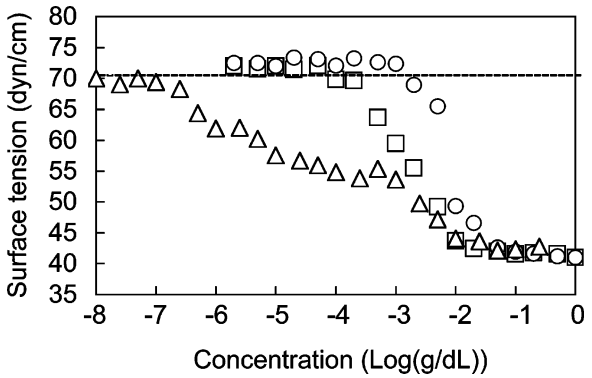



**Figure 4.** Surface tension of PIBr-*g*-PMPC in aqueous solution. ○, PIBr$_4$-*g*-PMPC17.8K; □, PIBr$_6$Ch$_1$-*g*-PMPC14.2K; △, PIBr$_3$Ch$_2$-*g*-PMPC14.9K.

**Surface Activity of Graft Copolymers.** Figure 4 shows the surface tension of graft copolymers in water as a function of polymer concentration. Changes in the surface tension are typically observed in the micelle formation of polymer amphiphiles. The cmc of the graft copolymers was evaluated from the change in surface tension. The cmc (g/dL) of each graft polymer was $8.2 \times 10^{-3}$ (PIBr$_4$-*g*-PMPC17.8), $2.0 \times 10^{-3}$ (PIBr$_6$Ch$_1$-*g*-PMPC14.2K), or $5.0 \times 10^{-4}$ (PIBr$_3$Ch$_2$-*g*-PMPC14.9K). The amount decreased with an increase in the number of cholesteryl groups in the graft copolymer. The polyphosphates of the graft copolymers act as hydrophobic polymers of the polymer surfactants. In the case of PIBr$_3$Ch$_2$-*g*-PMPC14.9K, two transitions were observed. The first transition and second transitions might be due to the intramolecular and intermolecular associations of the graft copolymers. The *cmc* can be controlled while retaining a high degree of surface activity by their well-controlled molecular architectures.

**Solution Property of Graft Copolymers in Water.** Micelle formations were also investigated by the fluorescence probe method. Figure 5 shows typical fluorescence spectra of ANS in an aqueous solution of the graft polymers at different concentrations. Weak fluorescence was observed in the ANS aqueous solution without any polymer. However, the intensity of the fluorescence of the ANS solution increased as the polymer concentration increased. In particular, the intensity of the fluorescence of ANS incorporated in PIBr$_3$Ch$_2$-*g*-PMPC14.9K was significantly higher than that of PIBr$_4$-*g*-PMPC17.8K.

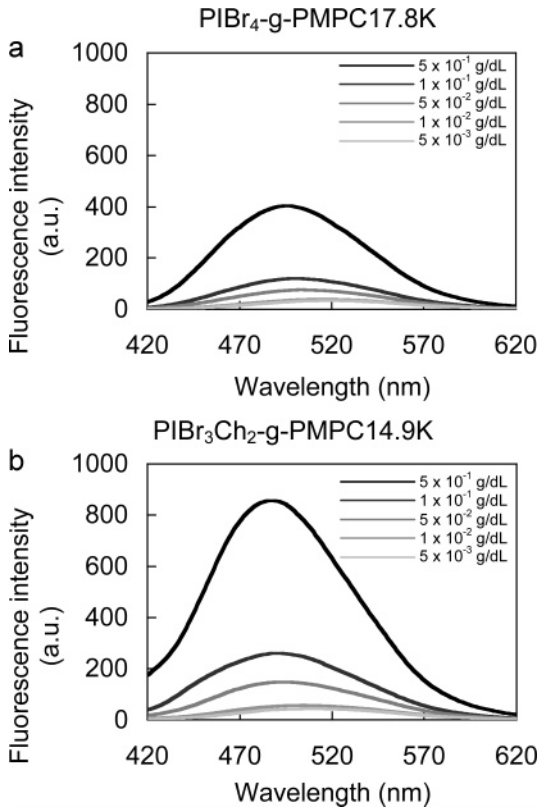



**Figure 5.** Fluorescence spectra of ANS incorporated in (a) PIBr$_4$-*g*-PMPC17.8K or (b) PIBr$_3$Ch$_2$-*g*-PMPC14.9K in aqueous solution.

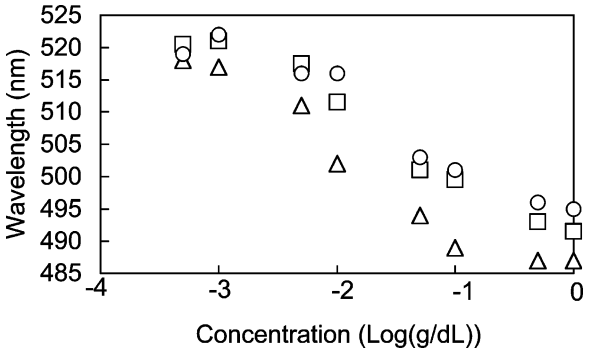



**Figure 6.** Concentration-dependence of maximum fluorescence wavelength of incorporated ANS. ○, PIBr$_4$-*g*-PMPC17.8K; □, PIBr$_6$-Ch$_1$-*g*-PMPC14.2K; △, PIBr$_3$Ch$_2$-*g*-PMPC14.9K.

Figure 6 shows the dependence of the maximum fluorescence peak wavelength of ANS in graft polymer aqueous solutions on its concentration. The behavior change in the wavelength of ANS in the polymer solutions was similar to that of their surface tensions. The maximum fluorescence wavelength of ANS is influenced by the polarity of the microenvironment[44] and is lower with lower polarity. Above *cmc*, the maximum fluorescence wavelength of ANS incorporated into PIBr$_3$CH$_2$-*g*-PMPC14.9K was 486 nm. At values lower than that, it was incorporated into PIBr$_4$-*g*-PMPC17.8K (495 nm). This result suggests that the hydrophobic association of graft copolymers is strongly influenced by the number of cholesteryl groups.

The typical hydrodynamic radii ($R_h$) of the solution for graft polymers above the transition point of the surface tension was measured by DLS. $R_h$ of PIBr$_3$Ch$_2$-*g*-PMPC14.9K was 7.5 nm.In other polymers, the level of light scattering was too low to evaluate the size of the polymer. MALLS analysis was performed to obtain further information about the nanoparticles for PIBr$_3$Ch$_2$-*g*-PMPC14.9K.

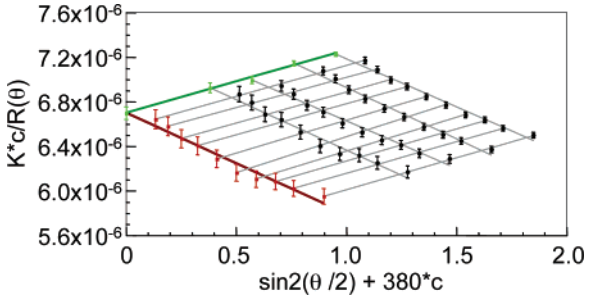



**Figure 7.** Zimm plot of associated $PIBr_3Ch_2$-*g*-PMPC14.9K. [Polymer] (g/dL) = 0.1, 0.15, 0.2, and 0.25.

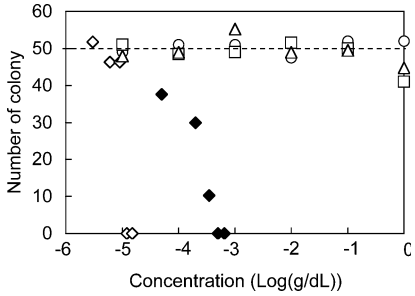



**Figure 8.** Number of v-79 cell colonies formed after contact with hydrated PCPG. ◇, ZDEC; ◆, ZDBC; ○, $PIBr_4$-*g*-PMPC17.8K; □, $PIBr_6Ch_1$-*g*-PMPC14.2K; △, $PIBr_3Ch_2$-*g*-PMPC14.9K. [Cells] = 50 cells/well.

Figure 7 shows the Zimm plot of $PIBr_3Ch_2$-*g*-PMPC14.9K at a concentration of 0.1−0.25 g/dL. The d*n*/d*c* of $PIBr_3Ch_2$-*g*-PMPC14.9K in aqueous solution was 0.119. Based on this plot, $M_w$ of the polymeric associate was $14.9 \times 10^4$. From the absolute molecular weight of $PIBr_3Ch_2$ and $^1H$ NMR analysis, the molecular weight of $PIBr_3Ch_2$-*g*-PMPC14.9K can be estimated at $6.7 \times 10^4$. Thus, the association number of the $PIBr_3Ch_2$-*g*-PMPC14.9K was 2.2.

The average polymer density ($\phi_g$) was calculated by $R_h$ and $M_w$ of the polymer by the equation

$$\phi_g = \frac{M_w}{N_A\left(\frac{4}{3}\pi {R_h}^3\right)}^{-1} \quad (3)$$

The density was estimated to be 14.0 wt %. In our previous report, the density of the polymer associate of the graft polymer without a cholesterol group was 8.4 wt %. The addition of a cholesteryl group to the polyphosphate backbone formed the compact polymer associate.

**Cytotoxicity of Graft Polymers.** Figure 8 shows the number of v79 cell colonies that formed after contact with graft polymers. When the cells were in contact with control compounds such as ZDEC and ZDBC, the number of colonies decreased and was completely reduced at 0.125 and 7.5 $\mu$g/mL, respectively. In contrast, no decrease in the number of colonies formed due to contact with graft polymers was observed when the polymer concentration was below 0.1 g/dL. This result indicates that graft polymer materials are quite safe. We have reported the noncytotoxicity of degradation products from polyphosphates and poly(MPC).[45] Consequently, the cytotoxicity of the degradation products would be low.

**Solubilizing an Anticancer Drug with Graft Polymers.** PTX is a highly hydrophobic drug and is barely soluble in water (water solubility = 0.3 $\mu$g/mL). Because of its poor solubility in water and many other acceptable pharmaceutical solvents, specific emulsifiers, such as Cremophor EL, are used to formulate PTX in commercial injection solutions. However,

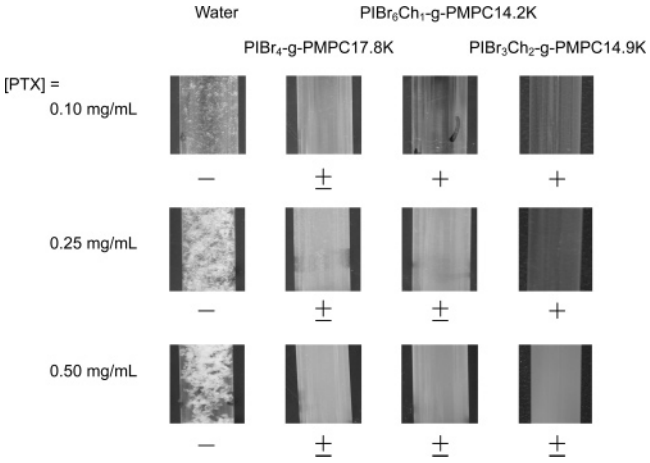



**Figure 9.** Photographs of paclitaxel aqueous solution containing graft polymers. +: Soluble (transparent), ±, cloudy; −, insoluble.

serious hypersensitive reactions have been reported in some individuals because the content of Cremophor EL, which is used in the PTX formulation, is significantly higher than in any other marketed drug.[46,47] Therefore, alternative dosage forms of PTX administration are needed to reduce the undesirable side effects induced by using Cremophor EL. Applications of liposomes,[48] mixed micelles,[49,50] parenteral emulsions,[51−53] cyclodextrin complexes,[54] and hydrotropic dendrimers[55] have been reported. Konno et al. reported that water-soluble MPC polymers could be applied for improving the solubility of PTX in aqueous media.[56] Furthermore, they reported that nanoparticles coated with MPC polymer were not recognized by macrophages. Due to the low toxicity of MPC polymers, the higher PTX dose with the polymers could be performed as compared with that with Cremophor EL.[57] These results describe that MPC polymers might be useful for drug delivery. We hypothesized that well-defined molecular design enables the production of a variety of amphiphiles and that biodegradability is an important factor in drug delivery. Figure 9 shows PTX in various MPC polymer solutions. In each case, ethanol was evaporated completely under reduced pressure. For $PIBr_4$-*g*-PMPC17.8K aqueous solutions, PTX was well dispersed compared with a polymer-free aqueous solution. However, 0.1 mg/mL PTX could not dissolve completely in 1.0 mL of a polymer solution containing 9.0 mg/mL of the polymer. In contrast, polymers containing cholesteryl groups can dissolve PTX completely. In particular, $PIBr_3Ch_2$-*g*-PMPC14.9K enhanced the solubility of PTX by about ~800 times.

After 0.1 mg/mL PTX was solubilized with $PIBr_3Ch_2$-*g*-PMPC14.9K (1 wt %), the yield of PTX collected in the solution (0.5 mL) by being passed through a filter (0.22 $\mu$m) was 94.6% as measured by UV spectrometry (246 nm). The $R_h$ of the polymer associate with PTX was 20.5 nm, larger than that of the polymer associate without PTX. After one week of storage at room temperature, the solution was still transparent and the collection yield of PTX was above 90%. The complexes of PTX and graft copolymers were then quite stable in aqueous media.

## Conclusion

This study describes a series of biodegradable graft copolymers as novel amphiphilic biomaterials. To control the hydrophobicity of a polyphosphate backbone, polyphosphates having cholesteryl groups were synthesized. Due to an increase in the composition of the cholesteryl groups in the graft polymers, it was made clear from the fluorescence probe and DLS analyses

that stable polymer associates were formed by hydrophobic interactions of cholesteryl groups. The cholesteryl groups were also important for improving the efficiency of graft copolymers as polymeric solubilizers for anticancer drugs such as PTX, and stable nanoparticles containing PTX were formed. Although many polymeric amphiphiles have been studied in similar applications, the number of well-designed graft polymers showing biodegradability and biocompatibility are still limited. Particularly, polyphosphates have recently attracted great interest in both fundamental and applied biomedical materials science.[25−28,45] The graft copolymers synthesized in this study can be applied for drug delivery systems and are important for the biomedical and pharmaceutical fields.

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