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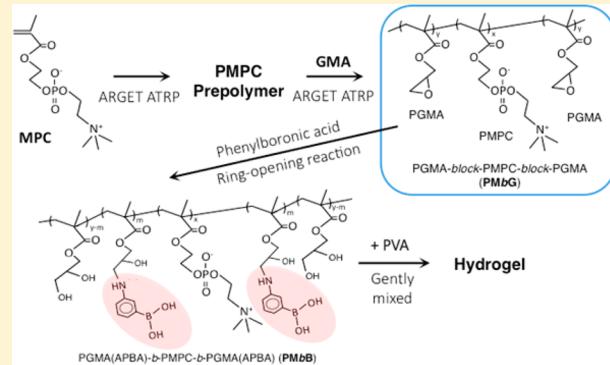
Amphiphilic Triblock Phospholipid Copolymers Bearing Phenylboronic Acid Groups for Spontaneous Formation of Hydrogels with Tunable Mechanical Properties

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 Supporting Information

ABSTRACT: ABA-type triblock copolymers composed of poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) and poly(glycidyl methacrylate) (PGMA) segments (PGMA-block-PMPC-block-PGMA (PMbG)) were synthesized by activator-regenerated by electron transfer atom transfer radical polymerization method. The PMPC segment provided water-solubility and the PGMA segments could react with nucleophile reagents for chemical functionalization. Phenylboronic acid (PBA) derivatives were bonded to PMbG via epoxide group to obtain PBA-connecting phospholipid polymer (PMbB). The PMbB formed hydrogels in aqueous medium spontaneously when it was mixed with poly(vinyl alcohol) (PVA) aqueous solution due to reaction between PBA groups in PMbB and hydroxyl groups in PVA. The mechanical properties of PMbB/PVA hydrogel were dependent on the length of PMPC segment and pH of polymer solution. According to control of both chemical structure and mechanical properties of the hydrogel, the PMbB is a good candidate for applying in biomedical fields as polymer matrices for cell encapsulation or protein immobilization.



1. INTRODUCTION

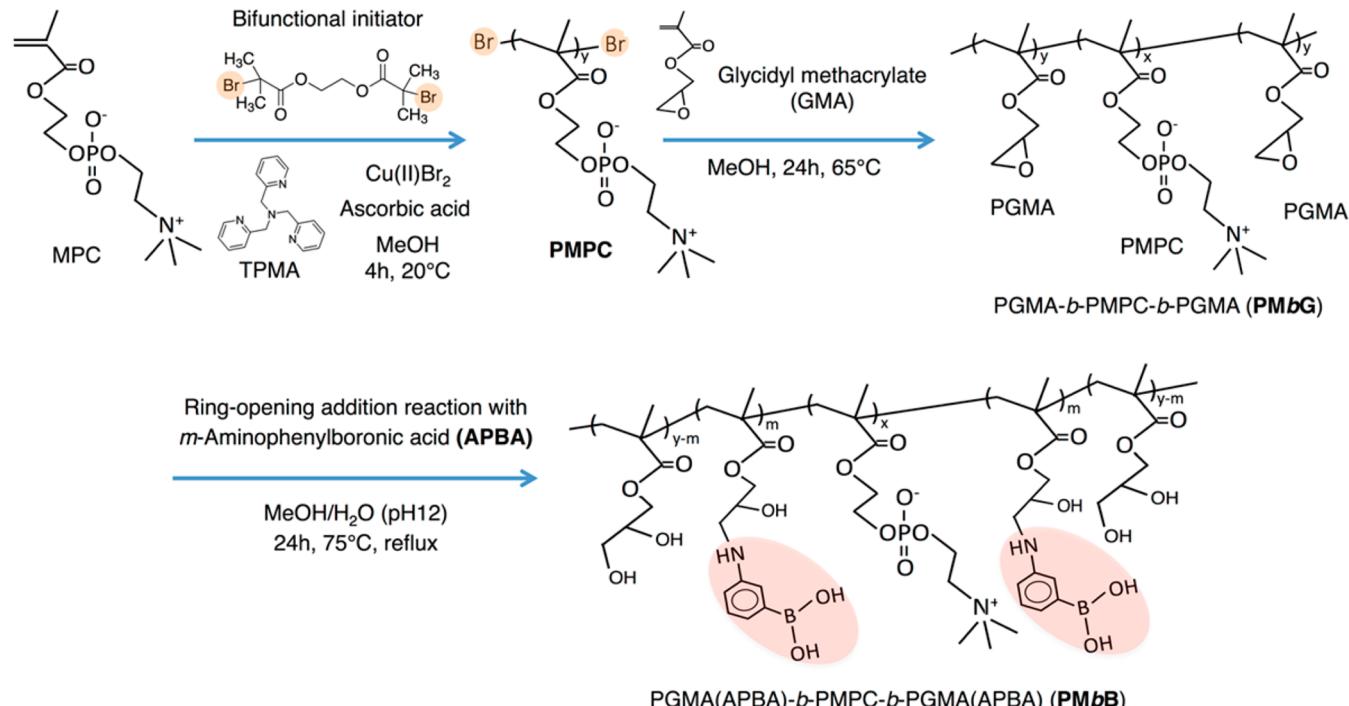
ABA-type triblock copolymers consisting of a water-soluble B segment and functional A segments have received widespread attention, as they can serve as nano-/microscale building-block molecules that can self-assemble in aqueous solutions into flower-structured micelles or polymersomes^{1,2} or form 3-dimensional viscoelastic hydrogels when the B segment acts as a cross-linker.^{3–7} The key advantage of employing well-defined ABA-type triblock copolymers over conventional random copolymers as hydrogel precursors is the precisely controllable length of each segment, which affects the distance between cross-links.^{8,9} The hydrophilic segment is considered an elastically active chain in the hydrogel matrix, which can impact the strength and stiffness of the hydrogel;^{10,11} shorter and harder elastic segments usually lead to hydrogels with high strength, while softer hydrogels can be obtained from polymers with long and flexible elastic chains. In addition, the defined length of the hydrophilic segment can also lead to improved homogeneity of the nanoscaled network and more precisely tunable hydrogel properties; this has recently become a requirement, as it could essentially affect cellular activities such as cellular proliferation or differentiation.^{12–14} To prepare well-defined block copolymers, atom transfer radical polymerization (ATRP) is a significantly important method and has been employed in the synthesis of a wide range of diblock and multiblock copolymers.^{15–18}

Phospholipid polymers, i.e., 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, have been proved to have high cytocompatibility, which is the resistance of protein adsorption and cell adhesion.^{19–21} Hydrogels constructed from MPC polymers for cell encapsulation could exhibit cell sustainability with a high survival rate²² and controllable cellular activity^{23,24} and could be easily fabricated as platforms for cell-to-cell interactions.²⁵ ATRP has been employed to prepare MPC polymers with practical control over the polymer chain length with a low polydispersity²⁶ to serve as the starting component for various materials, from thermoresponsive hydrogels²⁷ to polymeric stabilizers,²⁸ with applications from hydrophobic drug delivery to DNA condensation.^{29,30}

In this study, well-defined ABA-type triblock copolymers composed of poly(MPC) (PMPC) as the middle B segment were prepared. Rather than limiting the function of the A segments of the triblock copolymers, we used poly(glycidyl methacrylate) (GMA) segments, which contain the versatile epoxide group. The epoxide group in GMA can be easily functionalized by addition reactions with nucleophile groups such as amino, hydroxyl, and carboxyl groups, which will allow the polymers to be used for various applications

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Scheme 1. Synthesis of ABA-Type Triblock Phospholipid Copolymers

depending on the introduced functional group.^{31–33} PGMA-*block*-PMPC-*block*-PGMA (PMBG) is expected to have substantial cytocompatibility; however, conventional ATRP requires a large amount of metallic catalysts such as copper bromide that exert some undesirable influence on the cellular activity.³⁴ Hence, we decided to employ the recently developed method of activators regenerated by electron transfer (ARGET) ATRP, which reduced the amount of metallic catalyst to a few ppm in the presence of a natural reducing agent (e.g., ascorbic acid).^{35–37} The polymerization of extremely water-soluble MPC and poorly water-soluble GMA by conventional ATRP is difficult; nonetheless, the synthesis of PMbG was more facile using ARGET ATRP. To the best of our knowledge, this is the first example of the synthesis of a block MPC copolymer using the ARGET ATRP method.

PBA derivatives can produce stable but reversible complexes with diol compounds.³⁸ They are also widely used as cross-linking agents in cytocompatible hydrogels.^{24,39,40} As one example of the versatility of PMbG, water-soluble PMbG was functionalized with amino-containing PBA groups, introduced into the polymer via reaction with the epoxide group in the PGMA segments. The PBA group-connected triblock MPC copolymer (PMBB) was employed as a precursor for the formation of a hydrogel when mixed with poly(vinyl alcohol) (PVA) in aqueous medium. We also investigated the effects of the properties of PMbB, such as the length of the PMPC segment and the reactivity of the pH-sensitive PBA group to hydroxyl groups in PVA in different pH of the medium, on the properties of the PMbB/PVA hydrogels.

2. EXPERIMENTAL SECTION

2.1. Materials. MPC was obtained from the NOF Co., Ltd. (Tokyo, Japan), which was synthesized by a previously reported procedure.⁴¹ GMA, ethylene bis(2-bromoisobutyrate) (EBBiB), tris(2-pyridylmethyl)amine (TPMA), copper(II) bromide (CuBr₂), and ammonium 8-anilino-1-naphthalenesulfonate (ANS) were purchased

from Sigma-Aldrich (St. Louis, Missouri, USA). 3-Aminophenylboronic acid (APBA), L-ascorbic acid, and poly(vinyl alcohol) (PVA; degree of polymerization ~1000) were obtained from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). D-Sorbitol was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). All solvents and reagents were extra-pure grade and were used as received without further purification.

2.2. Instrumentation. For the characterization of the polymers, the compositions of monomer units were characterized by nuclear magnetic resonance (¹H NMR) spectroscopy (400 MHz NMR spectrometer; JEOL Ltd., Tokyo, Japan). The molecular weights and polydispersity were evaluated by gel permeation chromatography (GPC; Jasco system equipped with OHpak SB-804 HQ column, Shodex, Tokyo, Japan) using methanol/water (70/30 v/v) containing 10 mmol/L of lithium bromide (LiBr) as an eluent. A fluorescence spectrometer (FP-8500, Jasco Co. Ltd., Tokyo, Japan) was employed to examine the polymer structure in aqueous medium with a fluorescent probe.

For the characterization of the hydrogels, the morphology of a freeze-dried hydrogel was observed by scanning electron microscopy (SEM; SM-200, Topcon Co. Ltd., Tokyo, Japan) after coating by gold sputtering. The average hydrogel pore sizes were examined using ImageJ software (ImageJ, U.S. National Institutes of Health, Bethesda, MD). The hydrogel formation kinetics and hydrogel viscoelastic properties were measured using a rheometer (Rheograph-Micro, Toyo-seiki Co. Ltd., Tokyo, Japan). For kinetics measurements during gelation, a specific amount of polymer solution was simultaneously injected into an aluminum mold (dimensions, 16.0 × 45.0 × 2.6 mm³; total sample volume, 1.5 mL) that was fixed to the vibrating unit of the rheometer. The mixing blade (dimensions, 42.0 × 11.0 mm² containing open holes 3.0 mm in diameter; total surface area, 257 mm²), which was connected to a measuring unit, was immediately inserted into the gelation chamber, and the data were recorded. For measuring the hydrogel strength, completely prepared hydrogels were transferred into the metallic mold and attached to the rheometer. The smooth blade (dimensions: 40.0 × 10.0 mm²) was used for measuring the modulus. Detailed characterization procedures are reported in each section.

2.3. Synthesis of PMbG by ARGET ATRP. The synthesis of PMbG was conducted by ARGET ATRP in one pot, where the second

monomer was injected into the reaction chamber after polymerization of the first monomer was complete, employing a so-called “sequential addition” approach. In the first step, the MPC was polymerized to obtain PMPC prepolymer as follows. A stock solution of CuBr₂ was prepared by dissolving CuBr₂ (8.9 mg) in degassed methanol (MeOH) (1.0 mL) and was kept in a sealed container until used. The polymerization was performed by adding MPC (5.9 g), with EBBiB (72.0 mg) as a bifunctional initiator, TPMA (11.6 mg) as a ligand, and ascorbic acid (7.0 mg) as a reducing agent in a triple-neck round-bottom flask. Degassed MeOH was transferred to the flask. Argon was bubbled through the solution while vigorously stirring until all solids were dissolved. The flask was sealed, and three cycles of degassing and argon bubbling were performed. CuBr₂ solution (100 μL) was then injected into the mixture. The final molar composition of the polymerization reaction was MPC/EBBiB/Cu(II)Br₂/TPMA/ascorbic acid 100/1.0/0.020/0.20/0.20 (resulting in PMPC of an anticipated chain length of 100 monomer units). The polymerization was carried out under stirring in a refrigerator with a fixed temperature of 20 °C.

After 4 h, the polymerization of MPC was confirmed to be higher than 98% conversion, as judged by ¹H NMR spectroscopy (with methanol-*d*₄ as solvent) by observing the disappearance of the vinyl signals of MPC at 5.5 and 6.0 ppm (Supporting Information, Figure S1). Under a continuous flow of argon, degassed GMA (1.42 g) was injected into the reaction flask by a gastight syringe, followed by three cycles of degassing and argon bubbling. The reaction flask was transferred to an oil bath at 65 °C on a stirrer, and secondary polymerization was allowed to continue for 24 h until ¹H NMR data indicated no further monomer conversion. The degree of polymerization of GMA was determined from the ¹H NMR data by comparing the characteristic peaks of vinyl group (δ at 5.5 and 6.0 ppm) to the characteristic peaks of the epoxide ring (δ at 2.6 and 2.8 ppm). The raw polymer product was cooled down to room temperature and precipitated from acetone/tetrahydrofuran (80/20 v/v) and dried in a desiccator until no weight change was observed. The purity of the polymer was confirmed by ¹H NMR measurements focusing on the disappearance of the vinyl signal of the monomers. The final polymer product was obtained as a pale brown powder (6.5 g) and was kept in a sealed container at room temperature until used. Other PMbGs with different lengths of the PMPC segment were also prepared by the same procedure by changing the proportion of MPC to initiator in the ratio $X/1.0/0.020/0.20/0.20$ ($X = 50$ and 200; with the anticipated MPC unit per chain equal to 50 and 200 units, respectively). Preparation of the PMbG triblock copolymer by ARGET ATRP is illustrated in Scheme 1. All the prepared polymers were characterized by ¹H NMR using ethanol-*d*₆ to indicate the actual ratio of MPC units to GMA units in the polymer chain.

2.4. Reaction of PMbG with APBA. The reaction of APBA with the PGMA segments in PMbG was carried out as follows. PMbG100 (PGMA₉-block-PMPC₁₀₀-block-PGMA₉) (1.0 g) was dissolved in 10 mL MeOH/H₂O (pH 12) (50/50 v/v) in a 20 mL round-bottom flask, and argon gas was bubbled through the solution for 10 min before the addition of APBA (2.5 g) predissolved in MeOH (5.0 mL). The mixture was then degassed by sonication. After all solids disappeared, the reaction chamber was transferred to an oil bath and allowed to react at 75 °C under reflux and stirring for 24 h. The reaction was stopped after the characteristic peaks at 2.6 and 2.8 ppm from the epoxide of GMA disappeared in the ¹H NMR spectra. Methanol in the reaction mixture was evaporated by a rotary evaporator, and the remaining aqueous polymer solution was transferred to a Spectra/Por dialysis tube (MWCO 3.5 kDa, Funakoshi Co., Ltd, Tokyo, Japan) and dialyzed to remove unreacted reagents against 10 mM NaOH, 10 mM HCl, and distilled water, successively. The final polymer was lyophilized to obtain PMbB (0.82 g; 82.3% yield). PMbB50 and PMbB200 (with PMPC chain lengths equal to 50 and 200 units, respectively) were also prepared by the same procedure.

2.5. Analysis of the solubility of PMbB in aqueous medium. Buffered solutions were typically prepared in a pH range of 7.2 to 10.4 by two different buffered systems. Tris-based buffer solutions were employed for pH 7.2–9.0, while sodium carbonate/bicarbonate-based

buffer solutions were prepared for pH 9.0–10.4. A small amount of PMbB was dissolved in each pH buffer solution containing 1.0×10^{-5} M ANS until the final concentration of the polymer was 0.010 mg/mL. The fluorescence spectra were obtained with an excitation wavelength of 350 nm.

2.6. Gelation of PMbB with PVA. PMbBs were dissolved in aqueous pH buffer solutions by stirring at room temperature. PVA solutions were prepared by mixing PVA with pH buffer solutions and allowed to dissolve at elevated temperature by autoclave. All polymer solutions were sonicated prior to further experiments. The gelation conditions of the PMbB/PVA hydrogels were studied by mixing solutions of PMbB and PVA with various concentrations (from 1.0 to 11% (w/v)) and pH (from pH 7.4–10.2). Preliminary observations to determine whether the hydrogel was formed or not were conducted by the test tube inversion method after mixing the polymer solutions by pipetting.

The rheological characteristics of the PMbB/PVA hydrogels were typically obtained by oscillatory shear measurements using a rheometer. Two characteristics were considered: the first was the gelation kinetics of PMbB/PVA hydrogel formation, and the second was the effects of the chemical composition of PMbB and the pH of the medium on the storage modulus of the PMbB/PVA hydrogels. The procedures used in these evaluations are described in detailed as follows. To analyze the gelation kinetics, 10% (w/v) of PMbB and 10% (w/v) of PVA were separately prepared in pH buffered solution. The polymer solutions were simultaneously injected into a rheometer mold followed by the insertion of a mixing blade. The measurement began immediately with a constant strain (0.20 mm) and vibrating frequency (20 Hz).

The dependence of the hydrogel strength on the type of PMbB and the pH was examined by the same rheometer; however, the smooth blade was applied for this measurement. Mixtures of PMbB/PVA were thoroughly mixed by pipetting and were allowed to form hydrogels overnight at room temperature. The prepared hydrogels were transferred to a rheometer mold and allowed to settle for another 1 h in a humid environment to avoid dehydration. The samples were then measured by rheometer with constant conditions as described above. Each test was continued until the storage modulus and loss modulus became constant. pH dependence plots of the storage modulus of each PMbB/PVA hydrogel were obtained.

3. RESULTS AND DISCUSSION

3.1. Characteristics of PMbG. The successful synthesis of the triblock copolymers, PMbG100, by ARGET ATRP was confirmed by ¹H NMR and GPC. The number-average molecular weight (M_n) and polydispersity (D) were determined by GPC using 10 mM lithium bromide (LiBr) in a MeOH/water (70/30 v/v) mixture as the eluent, as shown in Figure 1. As indicated by GPC, both PMPC as a prepolymer for

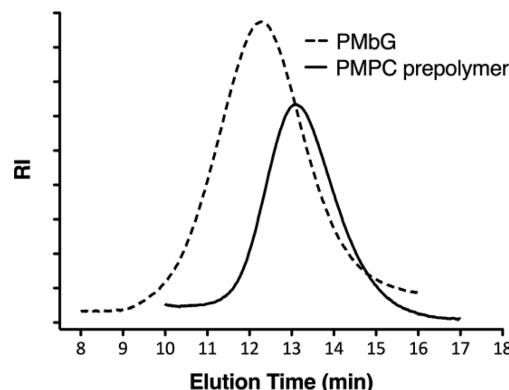


Figure 1. Representative GPC curves of the PMPC prepolymer and PGMA-block-PMPC-block-PGMA (PMbG).

Table 1. Synthetic Results of Polymers

polymers	composition of monomer units			in units-per-chain ^b			yield (%)	molecular weight ^c	
	MPC	GMA	APBA	MPC	GMA	APBA		M_n (10 ³)	M_w/M_n
PMBG50	73.5	26.5	—	50	20	—	98.7	5.80	1.21
PMBB50	73.5	11.8 ^c	14.7	50	10 ^d	10	86.6	5.80	1.20
PMBG100	84.7	15.3	—	100	18	—	96.1	7.40	1.43
PMBB100	84.7	6.6 ^c	8.7	100	8 ^d	10	82.3	7.50	1.24
PMBG200	91.7	8.3	—	200	18	—	97.1	13.4	1.64
PMBB200	91.7	4.2 ^c	4.1	200	9 ^d	9	91.0	13.4	1.24

^aCalculated from ¹H NMR results using EtOH-d6 as solvent. ^bCalculated from molar percentage based on assumption that the expected PMPC units-per-chain was achieved. ^cAssumed to be amount of epoxy group that loss or converted to be free diol group during modification process, calculated by subtracting the molar percentage of the PBA moiety in PMB_B from epoxy group in PMB_G. ^dAssumed to be amount of epoxy group that loss or converted to be free diol group during modification process, calculated by subtracting the number of units of PBA moiety in PMB_B from epoxy group in PMB_G. ^eExamined by GPC using MeOH/water (70/30 v/v) containing 10 mM LiBr as eluent.

preparing the PMB_G and PMB_B exhibited a unimodal elution curve. The curve of PMB_B is at an earlier elution time than that of PMPC, verifying that the extension of the PGMA segment from the PMPC chain was successful. The PMPC segment had a reasonably low polydispersity ($D = 1.16$), while the PMB_G with the shorter PMPC segment had a D of 1.21, and the PMB_G with the longer PMPC segment showed a slightly larger D of 1.43. Representative ¹H NMR spectra with labels of the relevant signals from each step of the synthesis are shown in the Supporting Information (Figure S2). The characteristic peaks attributed to the PMPC and PGMA segments in PMB_G were observed (δ in ppm: 2.56–2.70, 2.76–2.93, 3.15–3.32), and the integrals were used to calculate the unit composition of the block copolymer; the ratio of MPC units to GMA units in PMB_{G100} were determined to be 84.7 mol % to 15.3 mol %, respectively.

The modification of PMB_G with APBA was carried out by a conventional addition reaction under alkaline conditions (pH 12) at 75 °C. The success of the reaction was confirmed by ¹H NMR; the synthesized PMB_B exhibited the characteristic peaks of the PBA group (δ in ppm: 6.45–6.79, 6.92–7.15, 7.61–7.88), and the characteristic peaks of GMA were greatly reduced (Supporting Information, Figure S2). When comparing the fraction of GMA units in PMB_G to the fraction of APBA in PMB_B, approximately half of the GMA units were estimated to react with APBA. Considering that the characteristic peaks of GMA disappeared, the GMA units may undergo side reactions, i.e., transesterification with water molecules and epoxide ring-opening by hydrolysis; hydrolysis is believed to be more favorable at high pH, as used in our synthesis.³⁰ As hydrolyzed GMA units contain free diols, PMB_Bs in high concentration or high density can spontaneously form gels; thus, PMB_Bs have to be kept in powder form *in vacuo* until use. The molecular weight and D of the PMB_Bs were confirmed by GPC measurements under the same conditions reported above. The GPC results exhibited a slight change in the molecular weight of PMB_B compared to the original PMB_G; however, this was assumed to be due to an inevitable interaction based on the PBA unit and the GPC column. Some synthetic results of the obtained PMB_Gs and PMB_Bs are summarized in Table 1.

3.2. Properties of PMB_B in Aqueous Medium. It is important to characterize PMB_B in aqueous medium, especially under different pHs, as the PBA group in PMB_B can interact with the hydroxyl group in PVA in its anionic state. Because there is a small amount of PBA available in PMB_B, a traditional titration technique to determine the pH in the medium, where

the PBA groups in PMB_B change to their charged state, was limited. However, as uncharged PBA is less polar and more hydrophobic at lower pH,⁴² we applied fluorescent spectroscopy with a hydrophobic probe (ANS) to detect the transition between the uncharged trigonal planar geometry and charged anionic tetrahedral structure of the PBA groups in PMB_B. ANS has been widely employed to indicate the polarity of polymers in aqueous medium, and can function in a broad pH range.^{43,44}

As shown in Figure 2, at a constant concentration of PMB_B in buffers with various pHs, the maximum fluorescence

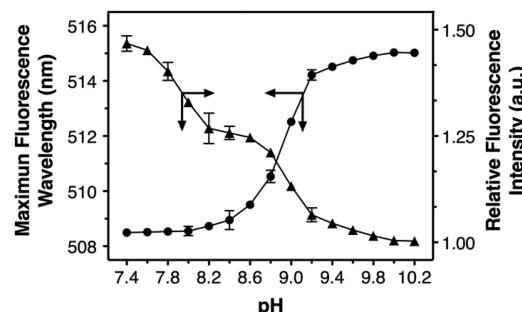


Figure 2. ANS fluorescent spectra position and intensity versus pH, demonstrating the change in the hydrophobicity of PMB_B in aqueous solution as a function of pH.

wavelength derived from the fluorescence spectra of ANS shifted from 508 nm (less polar environment) to 515 nm (more polar environment) from pH 7.4 to 10.2, with a reduction of the peak intensities. The maximum fluorescence wavelength of ANS increased drastically from pH 8.6 to 9.2 and reached a plateau at pH 9.4, which is comparable to the results in other literatures employed different approaches^{45,46} The midpoint of the chart, at approximately pH 8.9, was assumed to be the pH where the fraction of uncharged to charged PBA groups was almost unity. It can also be implied that the PMB_B became more hydrophilic at higher pH as a result of the conversion of the PBA groups in PMB_B to their charged anionic state, which would also lead to stronger bonding of PMB_B with PVA and a hydrogel with a higher stiffness.

3.3. Hydrogel Fabrication and Characterization. PMB_B/PVA hydrogels were formed by mixing various concentrations of PMB_B (2–10% (w/v)) with PVA solution at 10% (w/v) at varying pH (7.4–10.2) under gentle stirring at room temperature. Hydrogel formation was confirmed by a typical test tube inversion method (Supporting Information,

Figure S3). PM_bBs with different PMPC segment lengths (and different PBA compositions) can form hydrogels under different conditions. PM_bB50, PM_bB100, and PM_bB200 have PMPC segment lengths equal to 50, 100, and 200 units per chain, while the APBA was kept constant at approximately 10 units in a PM_bB polymer chain. The results from the ¹H NMR measurements confirmed the PBA group composition in PM_bB50, PM_bB100, and PM_bB200 to be 14.6%, 8.83%, and 4.64%, respectively. PM_bB50, with the highest composition of PBA, can form a hydrogel at physiological pH at concentrations as low as 5.0% (w/v), while PM_bB200 requires a high concentration (10.0% (w/v)) to form a self-standing hydrogel. The sol–gel phase separation of each PM_bB/PVA hydrogel is summarized in Figure 3 by inputting the conditions at which

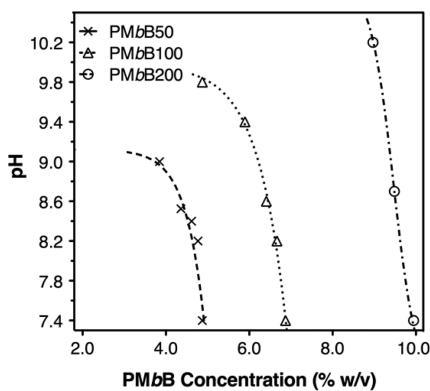


Figure 3. Sol–gel phase transition chart of the PM_bB/PVA hydrogels. The left side of the curve indicates the solution state, while the right side is the free-standing hydrogel state (constructed by nonlinear regression curve fitting with 95% confidence).

the hydrogel can be formed and performing nonlinear regression curve fitting with 95% confidence. The area on the left side of the phase separation line refers to the solution state, while the area on the right side indicates the formation of a self-standing hydrogel. However, the hydrogels observed by inverting the test tube varied from jelly like to hard, and thus the storage modulus (G') and loss modulus (G'') detected by oscillatory shear measurements were necessary to confirm the gelation for precise determination.

Dissociation of the PM_bB100/PVA hydrogel upon the addition of sugar molecules was observed by putting the hydrogel in sugar solution (0.10 M in water). The reversion of hydrogel to solution simultaneously took place at room temperature with gentle shaking. The hydrogel disappeared

after 30 min, as shown in the Supporting Information (Figure S3).

Figure 4 shows SEM images of lyophilized PM_bB/PVA hydrogels in the dried state. The PM_bB/PVA hydrogels were soaked in the buffer overnight before being lyophilized. A porous network was observed in all the PM_bB/PVA hydrogels. The pores in the PM_bB50/PVA and PM_bB100/PVA hydrogels were circular in shape with average pore diameters of $0.31 \pm 0.11 \mu\text{m}$ and $0.61 \pm 0.12 \mu\text{m}$, respectively. For the PM_bB200/PVA hydrogel, the SEM image showed a porous structure with an ellipsoidal shape that had a transverse diameter of $2.76 \pm 0.25 \mu\text{m}$ and a conjugate diameter of $0.66 \pm 0.14 \mu\text{m}$. The results confirmed that the size of the pores in the PM_bB/PVA hydrogels increased with the increasing molecular weight of the PMPC segment at consistent PGMA(APBA) segment. Hence, at a constant polymer concentration, the desired pore size and porosity of the hydrogels might be tuned by adjusting the composition of the elastically active chain and cross-linking point. The pore sizes of all the PM_bB/PVA hydrogels was assumed to be sufficient to allow the permeation of water-soluble molecules, as they were comparable to that of a conventional MPC polymer hydrogel composed of poly(MPC-random-*n*-butyl methacrylate-random-*p*-vinylphenyl boronic acid) (PMBV) and PVA.²⁴

3.4. Effects of the pH of the Medium on the Gelation Kinetics and Mechanical Properties.

The influence of pH on the gelation kinetics and mechanical properties of the PM_bB/PVA hydrogel system is very important, as the PBA groups in polymer side chain switch from an uncharged state to a charged anionic state in an alkaline environment.^{47,48} With increasing pH, the PBA groups in the polymer chain tend to be activated for reactions with the hydroxyl groups of PVA, which leads to a higher cross-link density and a harder gel. To quantify the influence of the pH on the gelation kinetics of the PM_bB/PVA hydrogel system, a series of polymer solutions in buffers with different pHs were prepared. The hydrogel precursor was identical for all samples (i.e., 10% (w/v) of PM_bB and 10% (w/v) of PVA, total volume of 1.5 mL). The results from the time-dependent gelation experiment (Figure 5) demonstrate cross-over of the storage modulus (G') and loss modulus (G'') of the PM_bB50/PVA hydrogel, which indicates the conversion of a viscous solution into a viscoelastic gel, known as the gel point⁴⁹ (Figure 5a). At pH 7.4, the gel point of the PM_bB50/PVA hydrogel was approximately 35 min; this value was maintained until pH 8.4, and then the time required to reach the gel point was gradually reduced to 15 min at pH 9.4. However, beyond pH 9.4, the mixture of PM_bB50 and PVA became a gel

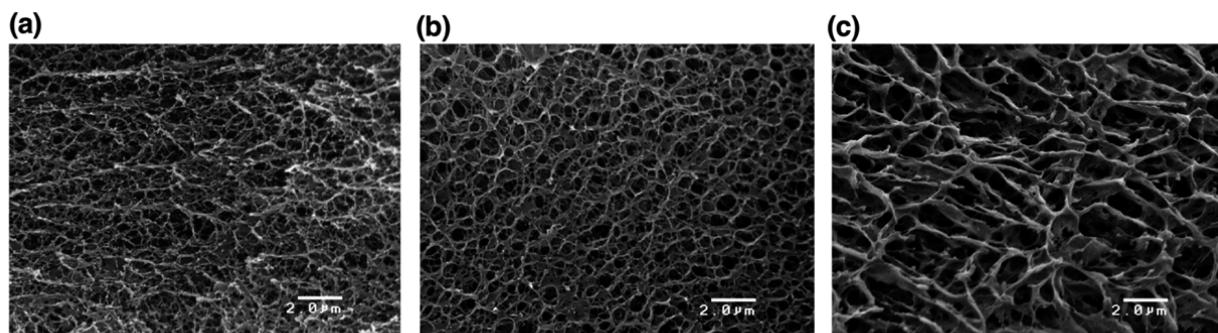


Figure 4. SEM images of the lyophilized PM_bB/PVA hydrogels with identical polymer concentrations: (a) PM_bB50/PVA hydrogel, (b) PM_bB100/PVA hydrogel, and (c) PM_bB200/PVA hydrogel (scale bar = $2 \mu\text{m}$).

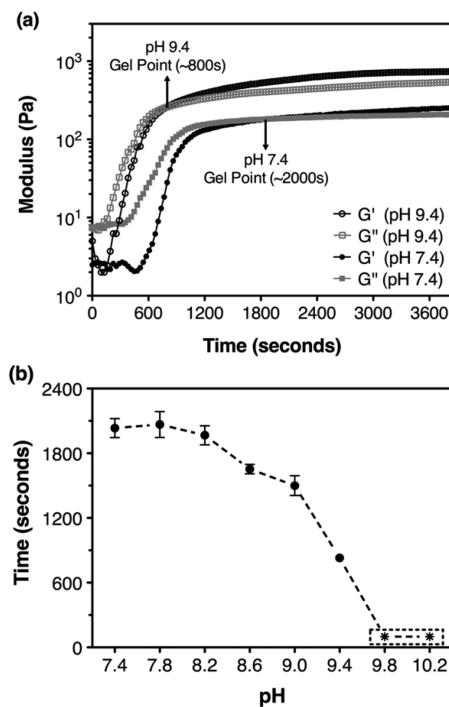


Figure 5. Gelation kinetics of the PMbB50/PVA hydrogel was strongly dependent on the pH. (a) Time-dependent change in the storage modulus (G') and loss modulus (G'') at pH 7.4 and 9.8. The crossover between G' and G'' indicates the gel point of the hydrogel. (b) Plot of the time required to reach the gel point as a function of the pH. At high pH, the gelation times are too short to be detected by rheometer and are represented with asterisks (*). The data represent the mean value, while the error bars stand for the standard deviation of three replicated samples.

immediately after mixing. The hydrogel adhered to the rheometer blade and resulted in fluctuating data, thus, at pH 9.8 and 10.2, we assumed that the gelation took place instantaneously (Figure 5b). For the hydrogel from PMbB100/PVA and PMbB200/PVA, the gel points from all pHs were greater than 3 h with a high deviation; therefore, we anticipate that there was some limitation in our real-time measuring equipment.

The results from the time-dependent gelation tests revealed the influence of pH on PMbB/PVA gelation. With increasing pH, anionic PBA groups in PMbB were expected to increase, which leads to more cross-linkable units and a faster reaction rate of the PBA groups with the hydroxyl groups of PVA. The results indicate that less time is required for gelation in alkaline environments.

The mechanical strength of the hydrogel is directly related to the number density of elastically active chains and the cross-link density, as described by the following elastic modulus equation:^{50,51}

$$G' = v_{el} k_B T; \quad \text{where } v_{el} = (f/2)\mu_{el}$$

v_{el} is the number density of elastically active chains, μ_{el} is the number of cross-links, f is the functionality, k_B is Boltzmann's constant, and T is the temperature.

For the PMbB/PVA hydrogels, the cross-link density predominantly depends on two factors: (1) the composition of PBA groups in the gel precursor (i.e., type and concentration of PMbB) and (2) the amount of anionic PBA groups, which is affected by the pH.

The curve in Figure 6a shows the storage and loss modulus at different pHs. Focusing on the PMbB50/PVA and PMbB100/

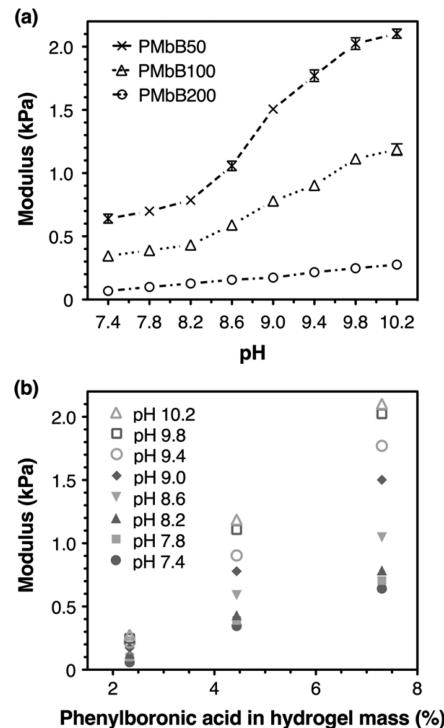


Figure 6. (a) Influence of the pH (from pH 7.4 to 10.2) on the storage modulus of the PMbB/PVA hydrogels. (b) Data showing the relationship between the hydrogel modulus and the percentage of PBA groups in the hydrogel mass. The data represent the mean while the error bars stand for the standard deviation of three replicated samples.

PVA hydrogels, the relationship between the storage modulus and the pH was sigmoidal in shape, with the modulus increasing with increasing pH. The first region from pH 7.4 to 8.2 represents the initial stage of the S-curve, in which the storage moduli did not significantly change. In the pH range from 8.2 to 9.8, the strength of the hydrogels sharply increased; the storage modulus of the PMbB50/PVA hydrogel increased to 2.00 ± 0.03 kPa at pH 9.8, which was 2.5 times that at pH 8.4 (0.80 ± 0.02 kPa), while the modulus increased from 0.40 ± 0.01 kPa to 1.10 ± 0.02 kPa in the case of the PMbB100/PVA hydrogel. The sigmoidal curve of the hydrogels reached a plateau at pH 9.8, and the storage modulus did not change much from pH 9.8 to 10.2. Meanwhile, for the PMbB200/PVA hydrogel, the amount of PBA in the hydrogel was substantially lower than in PMbB50 and PMbB100, hence, the change in the storage modulus of the hydrogel derived from PMbB200 did not exhibit a clear S-shape, but the storage modulus still gradually increased with increasing pH. The curves showing a comparison of the storage and loss modulus of the PMbB/PVA hydrogels at each pH are provided in the Supporting Information (Figure S4).

The mechanical modulus of the PMbB/PVA hydrogels in regards to the type of PMbB is shown in Figure 6b. The storage modulus of the hydrogel and the cross-linking density exhibited linear relationships that can be fitted to the elastic modulus equation. Additionally, considering each PMbB, storage modulus, and hydrogel morphology observed by SEM, the PMbB/PVA hydrogels with shorter PMPC segments possessed

higher strength with a tighter packing of polymer microstructures and a smaller pore size. Meanwhile, at an equivalent concentration of gel precursor, PMbB with longer PMPC segments resulted in softer gels with a looser polymer packing and larger pore sizes. By precise design of the polymer microarchitecture, the properties of the hydrogel on the macro-scale could be precisely controlled.

4. CONCLUSIONS

We prepared well-defined functionalizable ABA-type triblock copolymers with PMPC as the middle segment and PGMA as the terminal segments by ARGET ATRP, which allowed us to obtain PMbB with a low D and controllable PMPC segment length. The versatile epoxy group of the PGMA segments could be easily functionalized, and block copolymers bearing phosphorylcholine groups and PBA groups were successfully prepared. The gelation of PMbB with PVA is spontaneous under physiological conditions; meanwhile, the gelation kinetics and mechanical properties of the hydrogels are dependent on both the pH and length of hydrophilic block. The results show a sharp change in the mechanical characteristics between pH 8.2 and 9.8, as more PBA groups were converted to an active tetrahedral structure. At constant polymer concentrations (10% (w/v)), the storage modulus of the PMbB/PVA hydrogels could be tuned from 0.8 to 2.0 kPa, and the gelation time was varied from 30 min to seconds. The length of the elastic hydrophilic chain and the cross-linking density of the PMbB/PVA hydrogels showed linear relationships with the hydrogel strength. By considering both factors, controllably tunable MPC polymer hydrogels may be achieved. In addition, because of its high cytocompatibility (from the MPC unit) and its versatility (from the GMA moiety), PMbG is a good candidate for many applications in the field of functional biomaterials.

■ ASSOCIATED CONTENT

Supporting Information

All details in synthesis and characterization of the triblock copolymers, including time-dependence monomer conversion rate, ^1H NMR charts for each step of preparation, and dissociation of hydrogel. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Lee, J. S.; Feijen, J. *J. Controlled Release* **2012**, *161*, 473–483.
- (2) Pua, M. L.; Yoshitomi, T.; Chonpathompikunlert, P.; Hirayama, A.; Nagasaki, Y. *J. Controlled Release* **2013**, *172*, 914–920.
- (3) Kwon, K.-W.; Park, M. J.; Bae, Y. H.; Kim, H. D.; Char, K. *Polymer* **2002**, *43*, 3353–3358.
- (4) O’Lenick, T. G.; Jiang, X.; Zhao, B. *Langmuir* **2010**, *26*, 8787–8796.
- (5) Li, C.; Buurma, N. J.; Haq, I.; Turner, C.; Armes, S. P. *Langmuir* **2005**, *21*, 11026–11033.
- (6) Huynh, C. T.; Nguyen, M. K.; Lee, D. S. *Macromolecules* **2011**, *44*, 6629–6636.
- (7) Kissel, T.; Li, Y.; Unger, F. *Adv. Drug Delivery Rev.* **2002**, *54*, 99–134.
- (8) Aamer, K. A.; Sardinha, H.; Bhatia, S. R.; Tew, G. N. *Biomaterials* **2004**, *25*, 1087–1093.
- (9) Agrawal, S. K.; Sanabria-DeLong, N.; Tew, G. N.; Bhatia, S. R. *J. Mater. Res.* **2006**, *21*, 2118–2125.
- (10) Borzacchiello, A.; Ambrosio, L. In *Hydrogels: Biological Properties and Applications*, 2009 ed.; Barbucci, R., Ed.; Springer: New York, 2009; Vol. XI, p 10.
- (11) Larson, R. *The Structure and Rheology of Complex Fluids*; Oxford University Press, Inc.: New York, 1999.
- (12) Marklein, R. A.; Burdick, J. A. *Soft Matter* **2010**, *6*, 136–143.
- (13) Jeon, O.; Bouhadir, K. H.; Mansour, J. M.; Alsberg, E. *Biomaterials* **2009**, *30*, 2724–2734.
- (14) Seidlits, S. K.; Khaing, Z. Z.; Peterson, R. R.; Nickels, J. D.; Vanscoy, J. E.; Shear, J. B.; Schmidt, C. E. *Biomaterials* **2010**, *31*, 3930–3940.
- (15) Matyjaszewski, K. *Macromolecules* **2012**, *45*, 4015–4039.
- (16) Coessens, V.; Pintauer, T.; Matyjaszewski, K. *Prog. Polym. Sci.* **2001**, *26*, 337–377.
- (17) Lutz, J.-F.; Kirci, B.; Matyjaszewski, K. *Macromolecules* **2003**, *36*, 3136–3145.
- (18) Siegwart, D. J.; Oh, J. K.; Matyjaszewski, K. *Prog. Polym. Sci.* **2012**, *37*, 18–37.
- (19) Ueda, T.; Oshida, H.; Kurita, K.; Ishihara, K.; Nakabayashi, N. *Polym. J.* **1992**, *24*, 1259–1269.
- (20) Ishihara, K.; Takai, M. *J. R. Soc. Interface* **2009**, *6*, S279–S291.
- (21) Iwasaki, Y.; Ishihara, K. *Sci. Technol. Adv. Mater.* **2012**, *13*, 064101.
- (22) Aikawa, T.; Konno, T.; Takai, M.; Ishihara, K. *Langmuir* **2011**, *28*, 2145–2150.
- (23) Ishihara, K.; Xu, Y.; Konno, T. *Adv. Polym. Sci.* **2012**, *247*, 141–165.
- (24) Oda, H.; Konno, T.; Ishihara, K. *Biomaterials* **2013**, *34*, 5891–5896.
- (25) Gao, B.; Konno, T.; Ishihara, K. *Biomaterials* **2014**, *35*, 2181–2187.
- (26) Ma, I. Y.; Lobb, E. J.; Billingham, N. C.; Armes, S. P.; Lewis, A. L.; Lloyd, A. W.; Salvage, J. *Macromolecule* **2002**, *35*, 9306–9314.
- (27) Li, C.; Buurma, N. J.; Haq, I.; Turner, C.; Armes, S. P.; Castelletto, V.; Hamley, I. W.; Lewis, A. L. *Langmuir* **2005**, *21*, 11026–11033.
- (28) Thompson, K. L.; Bannister, I.; Armes, S. P.; Lewis, A. L. *Langmuir* **2009**, *26*, 4693–4702.
- (29) Licciardi, M.; Tang, Y.; Billingham, N. C.; Armes, S. P.; Lewis, A. L. *Biomacromolecules* **2005**, *6*, 1085–1096.
- (30) Lam, J. K. W.; Ma, Y.; Armes, S. P.; Lewis, A. L.; Baldwin, T.; Stolnik, S. *J. Controlled Release* **2004**, *100*, 293–312.
- (31) Ko, S.; Jang, J. *Biomacromolecules* **2007**, *8*, 1400–1403.
- (32) Padeste, C.; Farquet, P.; Potzner, C.; Solak, H. H. *J. Biomater. Sci., Polym. Ed.* **2006**, *17*, 1285–1300.
- (33) Reis, A. V.; Fajardo, A. R.; Schuquel, I. T. A.; Guilherme, M. R.; Vidotti, G. J.; Rubira, A. F.; Muniz, E. C. *J. Org. Chem.* **2009**, *74*, 3750–3757.
- (34) Studer, A. M.; Limbach, L. K.; Duc, L. V.; Krumeich, F.; Athanassiou, E. K.; Gerber, L. C.; Moch, H.; Stark, W. J. *Toxicol. Lett.* **2010**, *197*, 169–174.
- (35) Min, K.; Gao, H.; Matyjaszewski, K. *Macromolecules* **2007**, *40*, 1789–1791.
- (36) Pintauer, T.; Matyjaszewski, K. *Chem. Soc. Rev.* **2008**, *37*, 1087–1097.

- (37) Kwak, Y.; Magenau, A.; Matyjaszewski, K. *Macromolecules* **2011**, *44*, 811–819.
- (38) Springsteen, G.; Wang, B. *Tetrahedron* **2002**, *58*, 5291–5300.
- (39) Hendrickson, G. R.; Lyon, L. A. *Soft Matter* **2009**, *5*, 29–35.
- (40) Guan, Y.; Zhang, Y. *Chem. Soc. Rev.* **2013**, *42*, 8106–8121.
- (41) Ishihara, K.; Ueda, T.; Nakabayashi, N. *Polym. J.* **1990**, *22*, 355–360.
- (42) Cambre, J. N.; Sumerlin, B. S. *Polymer* **2011**, *52*, 4631–4643.
- (43) Collini, M.; D’Alfonso, L.; Baldini, G. *Protein Sci.* **2000**, *9*, 1968–1974.
- (44) Gussakovskiy, E. E.; Haas, E. *Protein Sci.* **1995**, *4*, 2319–2326.
- (45) Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Tetrahedron* **2004**, *60*, 11205–11209.
- (46) Li, X.; Pennington, J.; Stobaugh, J. F.; Schöneich, C. *Anal. Biochem.* **2008**, *372*, 227–236.
- (47) Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, I. K.; Lednev, C. S. *J. Am. Chem. Soc.* **2003**, *125*, 3322–3329.
- (48) Power, D. J.; Rodd, A. B.; Paterson, L.; Boger, D. V. *J. Rheol.* **1998**, *42*, 1021–1038.
- (49) Brinker, C. J.; Scherer, G. W. *Sol-gel science: the physics and chemistry of sol-gel processing*; Academic Press: Boston, MA, 1990; p 303.
- (50) Djabourov, M.; Nishinari, K.; Ross-Murphy, S. B. *Physical Gels from Biological and Synthetic Polymers*; Cambridge University Press: New York, 2013; p 111.
- (51) Mark, J. E. *Physical Properties of Polymers Handbook*, 2nd ed.; Springer: New York, 2007; Vol. XI, p 501.