Gelation Behavior of Poly(ethylene glycol) and Polycaprolactone Triblock and Multiblock Copolymer Aqueous Solutions

Soo Jin Bae,[†] Min Kyung Joo,[†] Yuri Jeong,[†] Sung Wook Kim,[‡] Woo-Kul Lee,[‡] Youn Soo Sohn,[†] and Byeongmoon Jeong*,[†]

Department of Chemistry, Division of Nano Sciences, Ewha Womans University, Daehyun-Dong, Seodaemun-Ku, Seoul, 120-750, Korea, and Laboratory for Biomaterials and Biointerfaces, Department of Chemical Engineering, Dankook University, Seoul 140-714, Korea

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ABSTRACT: We reported aqueous solutions of poly(caprolactone-*b*-ethylene glycol-*b*-caprolactone) (PCL−PEG−PCL) that underwent sol−gel−sol transition as the temperature increased (*Macromolecules* **2005**, *38,* 5260−5265). However, when the triblock copolymer aqueous solution (20 wt %), initially as a sol phase, was left at room temperature (20 °C), it turned into an opaque gel in 1 h. The crystallization of the PCL−PEG−PCL triblock copolymer in water was suggested to be responsible for such a kinetic aspect of the phase transition. In addition, PEG/PCL multiblock copolymers were synthesized by coupling the triblock copolymers using terephthaloyl chloride. Even though both PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions (20 wt %) instantaneously undergo a sol-to-gel transition upon injection into 37 °C water and their thermogels show a maximum modulus at around body temperature (35−42 °C), the multiblock copolymer shows a pronounced sol phase stability at room temperature. The fundamental difference in such phase behavior between triblock and multiblock copolymers seems to lie in their ability to form micelles at low temperature and high crystallizability of the low molecular weight PCL.

Introduction

In situ gel-forming polymers have recently been drawing attention as promising biomedical materials for minimally invasive therapy.[1,2] They enable pharmaceutical agents or cells to be easily entrapped and form a depot by a simple syringe injection at a target site, where the depot acts as a sustained drug delivery system or a cell-growing matrix for cells or stem cells.[3,4] Poly(ethylene glycol)/poly(propylene glycol) triblock and multiblock copolymers,[5,6] poly(ethylene glycol)/poly(butylene glycol) di- and triblock copolymers,[7] poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid) triblock and graft copolymers,[8] poly(ethylene glycol)/poly(propylene fumarate),[9] chitosan/glycerol phosphate,[10] and polyphosphazene[11] have been reported as a thermogelling polymer in water. The aqueous polymer solution is a low viscous sol at room temperature (20 °C) or lower and forms a gel at body temperature (37 °C).

Very recently, we reported poly(caprolactone-*b*-ethylene glycol-*b*-caprolactone) (PCL−PEG−PCL) as a new biodegradable thermogelling material.[12] The triblock copolymer has a powder morphology at room temperature, whereas the previous biodegradable thermogelling polymers such as poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid) triblock and graft copolymers, poly(ethylene glycol)/poly(propylene fumarate), and polyphosphazene have a sticky paste morphology. Thus, the PCL−PEG−PCL triblock copolymer is not only simple to transfer or weigh but also easily dissolved in water. The reconstitution/redissolution can be done in a couple of minutes by heating the polymer aqueous suspension above a melting point of the polymer (50 °C), followed by quick cooling in an ice bath.

Later, when the PCL−PEG−PCL triblock copolymer aqueous solution (20 wt %) was left at room temperature (20 °C) overnight, opaque gel formation was observed, even though it was previously defined as a sol. When the opaque gel (0.5 mL) formed at 20 °C was heated to 50 °C again for 30 s, followed by quickly quenching in an ice bath, it reversibly became a transparent free-flowing sol. When the transparent sol was injected into 37 °C water, it instantaneously became a gel in a second. Different mechanisms seem to be involved between the gel formed at 20 °C (low-temperature gel) and the gel formed at 37 °C (thermogel). An optimistic application of the PCL−PEG−PCL triblock copolymer as an injectable system is to keep the formulation as a solid powder in a vial. Then, an appropriate amount of water is added to the vial just before use, and the suspension is heated to 50 °C for 30 s, followed by quenching in an ice bath for 30 s to form a transparent solution. The solution is then injected subcutaneously or intramuscularly to form a gel depot. However, the fact that the formulation becomes an opaque gel at 20 °C when it is kept for more than 1 h might be a problem if the injection is not performed quickly after the redissolution of the formulation.

To understand the significant kinetic aspect of the PCL−PEG−PCL triblock copolymer aqueous solution, we investigated the polymer solution behavior using various instrumental methods such as UV−vis spectroscopy, rheometry, Raman spectroscopy, X-ray diffraction, optical microscopy, scanning electron microscopy, differential scanning calorimetry, ^{13}C NMR spectroscopy, and dynamic light scattering.

The crystallizability of polycaprolactone (PCL) decreases as the molecular weight of PCL increases.[13] Therefore, the triblock copolymers were coupled using terephthaloyl chloride to prepare a PEG/PCL multiblock copolymer which is expected to be less crystallizable due to the high molecular weight and terephthaloyl groups among the polycaprolactone. The physicochemical characteristics of the PEG/PCL multiblock and PCL−PEG−PCL triblock copolymer aqueous solutions were compared.

[†] Ewha Womans University.
[‡] Dankook University.
* Corresponding author: e-mail bjeong@ewha.ac.kr, Fax 82-2-3277-3419.

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Materials and Experimental Methods

Materials. ϵ-Caprolactone, stannous octoate, poly(ethylene glycol) (PEG) (MW = 1000), 1,6-diphenyl-1,3,5-hexatriene, terephthaloyl chloride, and anhydrous toluene were used as received from Aldrich. Triethylamine was dried over potassium hydroxide before use.

Multiblock Copolymer Synthesis. The PCL−PEG−PCL triblock copolymer was prepared by ring-opening polymerization of caprolactone in the presence of PEG as an initiator and stannous octoate as a catalyst.[12,14] To synthesize the PEG/PCL multiblock copolymer, the PCL−PEG−PCL (1000−1000−1000) triblock copolymer (10.0 g, 3.3 mmol) was dissolved in anhydrous toluene (80 mL), and the solvent was distilled off to a final volume of 30 mL to remove the residual water adsorbed to the polymer. Terephthaloyl chloride (0.694 g, 3.3 mmol) and triethylamine (1.41 mL, 10.12 mmol) were added to the reaction mixtures and stirred at 60 °C for 24 h. The product was precipitated into diethyl ether. The polymer was redissolved in methylene chloride, filtered, and then precipitated by slowly adding diethyl ether. The residual solvent was removed under vacuum.

^{1}H NMR ($CDCl_3$) of PEG/PCL multiblock copolymer:[15,16] δ 1.35 ($-OCH_2CH_2\boldsymbol{CH}_2CH_2CH_2CO-$), δ 1.62 ($-OCH_2\boldsymbol{CH}_2CH_2\boldsymbol{CH}_2CH_2CO-$), δ 2.30 ($OCH_2CH_2CH_2CH_2\boldsymbol{CH}_2CO$), δ 3.60 ($-O\boldsymbol{CH}_2CH_2-$), δ 4.06 ($-O\boldsymbol{CH}_2CH_2CH_2CH_2CH_2CO-$), δ 4.22 ($-CH_2\boldsymbol{CH}_2O-COCH_2CH_2CH_2CH_2CH_2CO-$), δ 4.35 ($-C_6H_4-CO-O\boldsymbol{CH}_2CH_2-CH_2CH_2CH_2CH_2CO-$), 8.10 ($-\boldsymbol{C}_6\boldsymbol{H}_4-$).

Gel Permeation Chromatography. The gel permeation chromatography system (Waters 515) with a refractive index detector (Waters 410) was used to obtain the molecular weights and molecular weight distributions of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers. Tetrahydrofuran was used as an eluting solvent. The PEGs in a molecular weight range of 400−20000 Da were used as the molecular weight standards. Styragel HMW 6E and HR 4E columns (Waters) were used in series.

NMR Study. A 250 MHz NMR spectrometer (9503DPX; Bruker) was used for ^{1}H NMR (in $CDCl_3$) to study composition of the polymers, and a 500 MHz NMR spectrometer (Unity-inova 500 MHz; Varian) was used for ^{13}C NMR to see spectral changes of the PEG/PCL multiblock copolymer (20 wt % in D_2O) as a function of temperature.

Sol−Gel Transition. The sol−gel transition was determined by the test tube inverting method.[17,18] The 4 mL vials (diameter 1.1 cm) containing 0.5 mL of the PCL−PEG−PCL triblock or PEG/PCL multiblock copolymer aqueous suspension were heated to 50 °C in a water bath for 30 s to melt the polymers and were quenched in an ice bath for 30 s. The polymer solutions were immersed in a bath at 10 °C for 2, 10, 20, 30, and 60 min to investigate the annealing effect. The transition temperatures were determined by a flow (sol)−no flow (gel) criterion when the vial was inverted with a temperature increment of 1 °C per step. Two minutes was maintained at each step. The same thermal history was given for the sample during the heating for the comparative purpose. The transition temperature in the phase diagram is an average of three measurements per each point.

Dynamic Mechanical Analysis. The sol−gel transition of the polymer aqueous solution was also investigated by dynamic mechanical analysis (rheometer RS 1; Thermo Haake).[19,20] The aqueous polymer solution (20 wt %) was placed between parallel plates of 25 mm diameter and a gap of 0.5 mm at 10 °C for 20 or 60 min. The data were collected under a controlled stress (4.0 dyn/cm^2) and a frequency of 1.0 rad/s. The heating rate was 0.5 °C/min.

Raman Spectroscopy. PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solution (20 wt %) were analyzed as a function of time by a Raman spectrometer (Lab Ram HR; Jobin-Yvon) at room temperature. The 514.5 nm of Ar ion laser was used for excitation. The slit width was 100 μm, and the exposure time was 120 s. All spectra were corrected for contribution from the quartz container. Estimated uncertainty of the peak frequencies was ± 1 cm^{-1}.

X-ray Diffraction Analysis. The PCL−PEG−PCL triblock copolymer aqueous solution (20 wt %) stayed at room temperature for 60 min to form a turbid gel. The X-ray diffraction data of the turbid gel were recorded with a Rigaku RINT2200 diffractometer using Cu Kα radiation at a scanning rate of 1°/min at room temperature (20 °C). The X-ray diffraction data of PCL (MW = 1250) and PEG (MW = 1000) were also studied for comparison.

Polarized Optical Microscopy. The polymer aqueous solution (20 wt %) was placed between two slide glasses, and a change in morphology was investigated using a polarized optical microscope (Olympus; Bh-753pw). The microscopic image was photographed at 0, 10, 35, and 60 min at room temperature. Then, the slide glass was heated at 50 °C for 30 s in the oven and quenched in the refrigerator for 30 s. And then, the morphology was also compared. Finally, the polarized optical microscopic images of the instantaneously formed gel by heating the slide at 37 °C were also compared.

Scanning Electron Microscopy. The polymer aqueous solution (1.0 wt %) was deposited on the cupper grid, and water was evaporated in air at room temperature for 48 h. The morphology was investigated using a scanning electron microscope (JSM-6700F; JEOL) after coating the sample with platinum. Resolution was 1.0 nm.

Differential Scanning Calorimetry. PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions (20 wt %) were prepared and kept at room temperature (20 °C) for 1 h. A differential scanning calorimeter (Diamond DSC; Perkin-Elmer) was used to study the heat exchange of an opaque PCL−PEG−PCL triblock copolymers gel (about 5.0 mg) formed at 20 °C and the PEG/PCL multiblock copolymer aqueous solution (5.0 mg) in a temperature range of 20−60 °C with a heating rate of 5.0 °C/min. The neat PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers were also studied in the same temperature range with a heating and cooling rate of 5.0 °C/min.

Dynamic Light Scattering. An apparent size of the PEG/PCL multiblock copolymer was studied by a dynamic light scattering instrument (Zetasizer nano ZS; Malvern) as a function of temperature at 1.0 and 13.0 wt %. The PEG/PCL multiblock copolymer aqueous solution was prepared in the same way with the PCL−PEG−PCL triblock copolymer aqueous solution to compare the solution behavior. The aqueous polymer suspension was heated to 50 °C for 30 s, followed by quenching in an ice bath for 30 s to form a transparent solution. During the measurement, the polymer solution was equilibrated at the designated temperature for 10 min. A He−Ne laser operating at 633 nm was used as a light source. Measurements of scattered light were made at an angle of 90° to the incident beam. The results of dynamic light scattering were analyzed by the regularized CONTIN method. The decay rate distributions were transformed to an apparent diffusion coefficient. From the apparent diffusion coefficient, the apparent hydrodynamic radius of the polymer or polymer aggregates can be obtained by the Stokes−Einstein equation.

Results and Discussion

The PCL−PEG−PCL triblock copolymer was prepared by ring-opening polymerization of ϵ-caprolactone on the PEG in the presence of stannous octoate as a catalyst.[12,14] In the case of α,ω-dihydroxyalkane, the two hydroxyl end groups show the same reactivity when the number of methylene units between the hydroxyl groups is over 6.[21] PEG (MW = 1000) has 22.7 ethylene glycol repeating units between two hydroxyl end groups; the principle of equal reactivity could be valid. Thus, the PCL blocks in the PCL−PEG−PCL were assumed to have the same molecular weight. The multiblock copolymer was prepared by coupling the hydroxyl end groups of the PCL−PEG−PCL triblock copolymers using terephthaloyl chloride. The synthetic scheme is shown in Figure 1.

The average number of ethylene glycol repeating units of PEG was assumed to be 22.7 because the molecular weight of

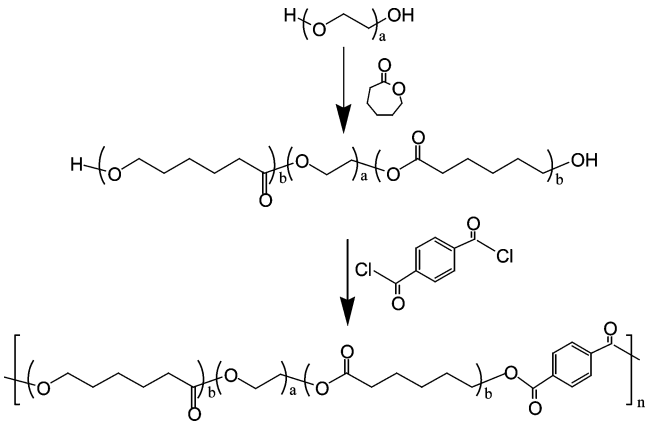

Figure 1. Synthetic scheme of a multiblock copolymer of PEG/PCL.

Table 1. List of Polymers Studied

polymer	M_n[b]	PDI (M_w/M_n)[b]
PEG[a]	1000	1.1
PCL−PEG−PCL triblock copolymer	2900	1.3
PEG/PCL multiblock copolymer	12700	2.3

[a] As received from Aldrich. [b] PDI: polydispersity index determined by gel permeation chromatography.

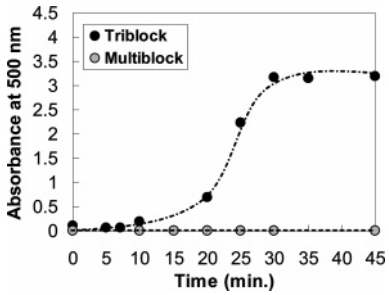

Figure 2. Change in the turbidity of the PCL−PEG−PCL triblock copolymer and PEG/PCL multiblock copolymer aqueous solutions (20 wt %) as a function of time when they were kept at room temperature.

starting PEG was 1000 Da. On the basis of the ^{1}H NMR peak area ratio at 3.6 ppm (PEG) to 2.3 ppm (methylene group next to PCL carbonyl group), the molecular weight of PCL−PEG−PCL triblock copolymer was calculated to be 1000−1000−1000 (total number-average molecular weight ∼ 3000).[15,16] The change in ^{1}H NMR spectra around 4.15−4.45 ppm also shows the progress of the reaction. A triplet at 4.22 ppm of the PCL−PEG−PCL triblock copolymer comes from a connecting ethylene group of PEG to PCL (−OCH_2***CH***$_2$$OCOCH_2CH_2CH_2CH_2CH_2CO$−). The two triplets in the ^{1}H NMR spectra of the PEG/PCL multiblock copolymer at 4.22 and 4.35 ppm come from ethylene glycol units connected to polycaprolactone (−CH_2***CH***$_2$$O-COCH_2CH_2CH_2CH_2CH_2CO$−) and caprolactone units connected to terephthaloly groups (−C_6H_4-CO-O***CH***$_2$-$CH_2CH_2CH_2CH_2CH_2CO$−), respectively. The peak at 8.1 ppm (phenyl group) also indicates the multiblock copolymer formation.

The progress of reaction was also followed by gel permeation chromatography. The starting PEG (MW = 1000 Da) appears at 18.0 min, while the PCL−PEG−PCL triblock copolymer and the PEG/PCL multiblock copolymer appear at 16.2 and 13.9 min in the chromatogram, respectively. The molecular weights of the PCL−PEG−PCL triblock and the PEG/PCL multiblock copolymers, determined by gel permeation chromatography, were 2900 and 12 700, respectively (Table 1).

Figure 2 shows the change in absorbance at 500 nm of PCL−PEG−PCL triblock and multiblock copolymer aqueous solutions

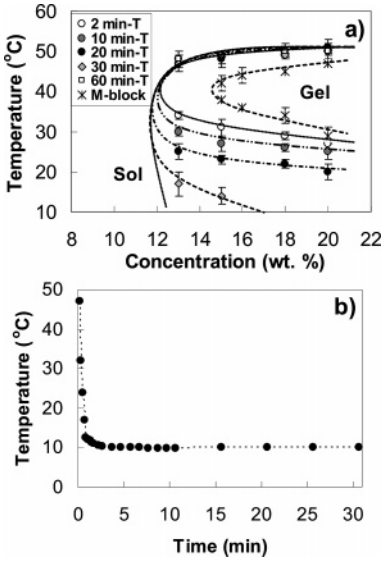

Figure 3. Phase diagram of the PCL−PEG−PCL triblock (2 min-T, 10 min-T, 20 min-T, 30 min-T, and 60 min-T), and PEG/PCL multiblock (M-block) copolymer aqueous solutions as a function of annealing time at 10 °C for 2, 10, 20, 30, and 60 min, respectively (a). The transition temperature was determined by the test tube inverting method. Each data point is an average of the three measurements. The temperature of the polymer solution was monitored by immersion of a thermometer in the vial containing polymer solution during the annealing process (b).

(20 wt %) as a function of time when they were kept at room temperature. The increase in absorbance at visible wavelength (500 nm) reflects the increase in turbidity.[22] Initially transparent triblock copolymer aqueous solutions became translucent in 20 min and opaque in 30 min. At the same time, viscosity of the solution apparently increased. In 30 min, the triblock copolymer solution became a viscous sol. Finally, it became a nonflowing opaque gel in 1 h even when the vial containing the polymer aqueous solution was inverted.

The sol−gel transition is observed for a polymer that has a delicate balance between hydrophilicity and hydrophobicity.[23,24] The change in the turbidity of PCL−PEG−PCL triblock copolymer aqueous solution (20 wt %) at room temperature was thought to be related to the crystallization of the triblock copolymer in water. However, the PEG/PCL multiblock copolymer aqueous solution remained as a transparent sol at room temperature even after 24 h.

To understand this phenomenon, sol−gel transition was studied as a function of annealing time at 10 °C. The phase diagrams of the triblock and multiblock copolymer aqueous solutions are shown in Figure 3. The transparent polymer solutions were prepared by heating the polymer/water mixture to 50 °C for 30 s, followed by quenching it in an ice bath (0 °C) for 30 s. The solutions were kept at 10 °C for 2, 10, 20, 30, and 60 min, and then the sol−gel transition was investigated with a temperature increment of 1 °C per step. The actual temperature of the polymer solution was monitored by immersing the thermometer in the polymer solution (Figure 3b). The temperature attained 10 °C in 2 min by the above procedure. As the temperature increased, the transparent sol-to-turbid gel transition occurred. At higher temperature, turbid gel-to-turbid sol transition occurred. Interestingly, the sol-to-gel transition temperatures of PCL−PEG−PCL triblock copolymer aqueous solutions were significantly influenced by the annealing history at 10 °C, whereas the gel-to-sol transition temperatures were not affected by the annealing history. The solutions that were annealed for 20 min at 10 °C showed about 10 °C lower sol-

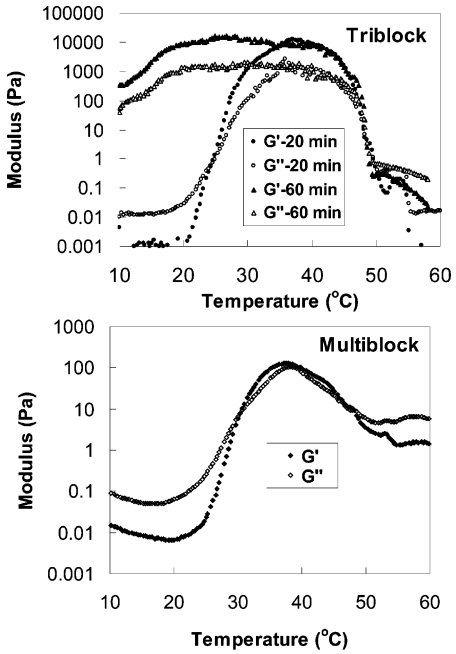

Figure 4. Change in modulus of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions (20 wt %) as a function of temperature. The PCL−PEG−PCL triblock copolymer aqueous solution (20 wt %) annealed at 10 °C for 20 min shows sol−gel−sol transition, whereas that annealed for 60 min shows gel melting at 50 °C, as the temperature increases.

to-gel transition temperatures than those of the solution annealed for 2 min in a concentration range of 13−20 wt %. The solution annealed for 60 min at 10 °C showed only a gel-to-sol transition at 50 °C because the solution already became a gel phase in this concentration range during the annealing procedure. On the contrary, the PEG/PCL multiblock copolymer aqueous solution kept transparency and stability as a sol at room temperature, and the sol−gel transition was reproducible, irrespective of the annealing procedure at 10 °C for 60 min. The critical gel concentration (15 wt %) above which a gel phase of the PEG/PCL multiblock copolymer exists was a little higher than that (13 wt %) of the PCL−PEG−PCL triblock copolymer. In addition, the gel window of the PEG/PCL multiblock copolymer, the range of temperature where a gel phase exists, was narrower than that of the PCL−PEG−PCL triblock copolymer.

Figure 4 shows changes in modulus of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions (20 wt %) as the temperature increases. The sol-to-gel transition at 20−30 °C was also accompanied by an abrupt increase in storage modulus (G') and loss modulus (G''). G' becomes larger than G'' in the gel region, and during the gel-to-sol transition at 45−50 °C, G'' becomes larger than G'. G' is an elastic component, and G'' is a viscous component of the complex modulus. From a mechanical point of view, a gel region can be defined by the zone where the elastic component (G') overwhelms the viscous component (G'').[25,26] In fact, the experimental determination of the sol−gel transition temperature is still a controversial issue. The $G'-G''$ crossover point is a function of frequency except for a material with a relaxation exponent (n) of 0.5.[27] The gel window of the current material determined by the test tube inverting method was 3−5 °C wider than that determined by the $G' = G''$ criterion.

The close value of G' and G'' in the gel phase of current polymers indicates the semisolid nature of the gel. The hydrogel should keep a three-dimensional mass without being dissolved in an excess amount of water.[28] The hydrogels of PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers were instantaneously formed when their aqueous solutions were injected into an excess amount of water at 37 °C. Also, the hydrogels were not dissolved out for several weeks. Maximal gel modulus (G'_{max}) appeared at 35−42 °C for both triblock and multiblock copolymers aqueous solution; however, G'_{max} of triblock copolymer was much larger than that of multiblock copolymer. Above the gel-to-sol transition temperature, both G' and G'' of the multiblock copolymer were kept at rather higher values than those of the triblock copolymer.

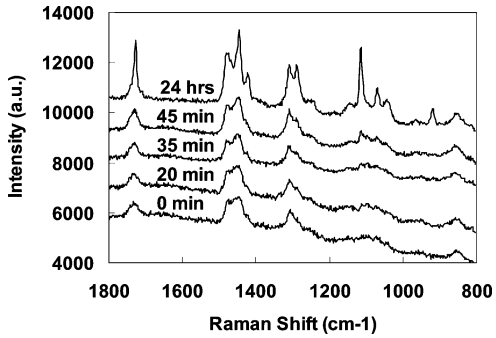

Figure 5. Change in Raman spectra of the PCL−PEG−PCL triblock copolymer aqueous solution (20.0 wt %) as a function of time at room temperature.

However, the G' of the PCL−PEG−PCL triblock copolymer aqueous solution annealed for 60 min at 10 °C was larger than G'' over 10−50 °C. Both the phase diagram (Figure 3) and the rheological analysis (Figure 4) suggest a gel phase of the sample in this temperature range. It showed only gel-to-sol transition at 50 °C as indicated by a sudden drop in G' and $G' < G''$.

From a practical point of view, the multiblock copolymer is more convenient than triblock copolymer in that it is stable at room temperature as a transparent solution. However, the triblock copolymer is better for the applications where a high modulus gel is needed. In some cell culture application, cells change their gene expression depending on the mechanical properties of the hydrogel.[29]

To understand the solidification process of the PCL−PEG−PCL triblock copolymer solution at room temperature, Raman spectra was recorded as a function of time at room temperature. The development of sharp Raman peaks during the polymer crystallization is well-known for polyethylene and polypropylene.[30,31] The Raman peaks of the PCL−PEG−PCL triblock copolymer aqueous solution were developed as a well-defined shape as the time elapsed, suggesting that crystallization of the polymer occurred (Figure 5). However, the Raman spectra (data not shown) of PEG/PCL multiblock copolymer did not change by the same procedure because the polymer solution kept its transparent sol state at room temperature.

The X-ray diffraction pattern of the turbid PCL−PEG−PCL hydrogel formed at room temperature for 60 min is shown in Figure 6. The two peaks at 21.3° and 23.9° coincided with those of PCL,[32] suggesting that the crystallization of the PCL is responsible for the gelation of the PCL−PEG−PCL triblock copolymer aqueous solution at room temperature.

The polarized optical microscopic images also suggest the crystallization of the PCL−PEG−PCL triblock copolymer at room temperature (Figure 7). T and M in the figure indicate triblock and multiblock copolymer, respectively. Time is the elapsed time after dropping the polymer aqueous solution (20 wt %) on the slide glass at room temperature. The significant crystalline phase was observed in the photograph of the PCL−PEG−PCL triblock copolymer aqueous solution taken after 35

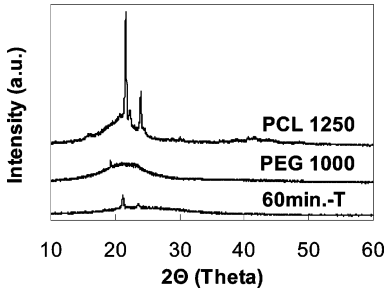

Figure 6. X-ray diffraction pattern of the PCL−PEG−PCL triblock copolymer hydrogel (60 min-T) formed by staying its aqueous solutions (20.0 wt %) at room temperature for 60 min. X-ray diffraction patterns of the PCL (MW = 1250) and PEG (MW = 1000) are shown for comparison.

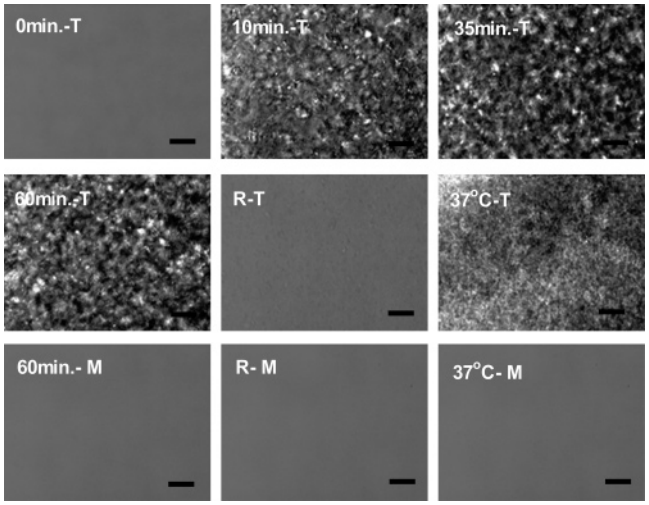

Figure 7. Polarized optical microscopic images of the PCL−PEG−PCL triblock and the PEG/PCL multiblock copolymer aqueous solutions (20.0 wt %) as a function of time. *N* min-T and *N* min-M indicate the images after *N* minutes at room temperature for triblock and multiblock copolymer aqueous solutions, respectively. R-T and R-M are the images prepared by a heating/quenching cycle of triblock and multiblock copolymer aqueous solutions, respectively. 37 °C-T and 37 °C-M are the images of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer hydrogels formed by direct heating at 37 °C. The scale bar is 280 μm.

min at room temperature. In 60 min, the polymer aqueous solution turned into an opaque gel, and the polarized optical microscopic image showed a bundle of the crystalline phase. The crystalline phase disappeared by the heating/quenching cycle described earlier (R−T in Figure 7). The polarized optical microscopic image of the PEG/PCL multiblock copolymer aqueous solution was the same over the same procedure because it stayed as a transparent solution at room temperature. Therefore, an image of the multiblock copolymer solution after keeping it at room temperature for 60 min is shown (60 min-M) in the figure. When both the PCL−PEG−PCL triblock and the PEG/PCL multiblock polymer aqueous solutions (20 wt %) were injected into 37 °C water, instantaneous gelation occurred. The polarized optical microscopic images of the hydrogels formed by direct heating the PCL−PEG−PCL triblock (37 °C-T in Figure 7) and PEG/PCL multiblock (37 °C-M in Figure 7) copolymer aqueous solutions (20 wt %) to 37 °C were compared. There is no apparent crystal-like phase for the multiblock copolymer (37 °C-M), whereas the triblock copolymer (37 °C-T) image shows some of crystalline morphology. The difference in the morphology might contribute to the much higher gel modulus of the triblock copolymer thermogel formed at 37 °C compared with the multiblock copolymer thermogel,

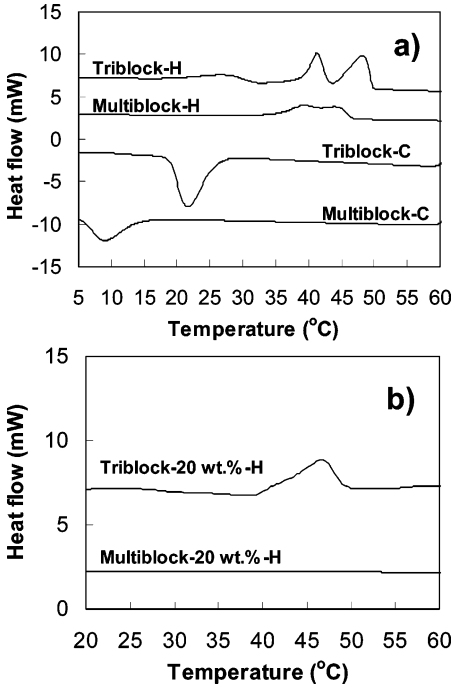

Figure 8. Differential scanning thermogram of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers as a neat powder (a) and the opaque gel prepared from the aqueous solution (20 wt %) of the PCL−PEG−PCL triblock copolymer at room temperature for 1 h and the PEG/PCL multiblock copolymer aqueous solution (20 wt %) (b). H and C indicate the first heating and the first cooling curves.

as shown in Figure 4. The fact that the hydrogel of PCL−PEG−PCL triblock copolymer formed at room temperature (low-temperature gel) showed a quite different morphology from the gel formed at 37 °C (thermogel) suggests that different mechanisms are involved in the gel formation of the PCL−PEG−PCL aqueous solution between room temperature and 37 °C. The low-temperature gel seems to be formed by crystallization of the polymer, and water is entrapped among the crystalline domains, whereas the thermogel seems to be formed by hydrophobic interactions and some of crystallization of the polymer.

The scanning electron microscope images of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers developed from its aqueous solution at room temperature show that the triblock copolymer has a fringed interwoven morphology with twisted and tightly packed strands, whereas the multiblock copolymer has a thicker fringed morphology (Figure S1).

Differential scanning calorimeter thermograms of the PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers show similar melting peaks at 40−50 °C in the heating curves (Figure 8a). The enthalpy of melting was 33.5 J/g for the PCL−PEG−PCL triblock copolymer and 24.3 J/g for the PEG/PCL multiblock copolymer. In the cooling curve, a larger enthalpy of crystallization at 23 °C observed for the PCL−PEG−PCL triblock copolymer (−41.4 J/g) than the PEG/PCL multiblock copolymer (−28.4 J/g) at 9 °C. The hysteresis of the melting peak and the crystallization peak comes from the slower crystallization rate than melting rate during the DSC experiment with the heating/cooling rate of 5.0 °C/min.

The PCL-PEG-PCL triblock copolymer opaque gel formed at room temperature showed an endothermic melting peak (T_m) at 46 °C during the heating, whereas PEG/PCL aqueous solution showed no detectable melting peak during the heating from room temperature to 60 °C (Figure 8b). The endothermic peak (17.1 J/g) in the heating curve of the opaque hydrogel coincided with

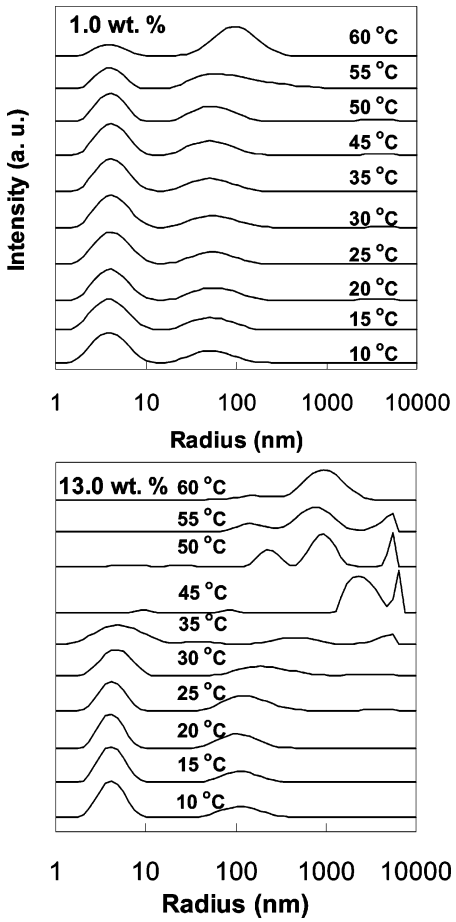

Figure 9. Apparent size of the PEG/PCL multiblock copolymer in water at low (1.0 wt %) and high (13.0 wt %) concentrations as a function of temperature.

the melting point of the neat PCL−PEG−PCL triblock copolymer. These thermograms suggest that crystallization tendency of the PCL−PEG−PCL triblock copolymer is higher than PEG/PCL multiblock copolymer at room temperature. And, such a trend is reflected in the aqueous solution of the polymer, resulting in a gelation of the PCL−PEG−PCL triblock copolymer aqueous solution, and a stable sol formation of the PEG/PCL multiblock copolymer aqueous solution at room temperature.

^{13}C NMR spectra of the PEG/PCL multiblock copolymer aqueous solution (20 wt % in D_2O: Supporting Information, Figure S2) as a function of temperature showed a pattern similar to the PCL−PEG−PCL triblock copolymer aqueous solutions.[12] As the temperature increased, the PEG peak collapsed and shifted to the down field, whereas PCL peaks sharpened. This fact suggests that molecular motion of PEG decreases, whereas that of PCL increases as the temperature increases. A downfield shift of the PEG peak has been reported in dehydration of PEG of the thermogelling polymers[33,34] and crystallization of the bulk PEG that accompany a decrease in the molecular motion.[35]

The apparent size and size distribution of block copolymers or their aggregates in water were investigated by dynamic light scattering.[36,37] At a low concentration (1.0 wt %), the bimodal distribution of the polymer size centered at 3.5−4.0 and 60 nm in radius were observed over 10−50 °C, whereas the dominant species shifted to a larger size of 60−100 nm at a higher temperature of 55 and 60 °C (Figure 9). Considering the fact that the unimer (single molecule) of PEG−PPG−PEG (2300−2000−2300; $M_n \sim 6600$) has a 2 nm radius in water,[5] the species at 3.5−4 and 60 nm in radius come from unimers and micelles of the PEG/PCL multiblock copolymer ($M_n \sim 12\,700$). This observation is in contrast to the PCL−PEG−PCL triblock copolymer in water that was reported earlier.[12] Above 25 °C, significant increases in the micellar radius from 6 nm (10 °C) to 20 nm (30 °C) were observed for PCL−PEG−PCL triblock copolymer. Micellar aggregation was suggested as a sol-to-gel transition (lower transition) mechanism of the PCL−PEG−PCL triblock copolymer. At a high concentration (13 wt %) of the PEG/PCL multiblock copolymer aqueous solution, unimers at 3.5−4 nm and micelles at 100 nm were observed at a low temperature. Above 35 °C, the micelles and unimers were aggregated to large particles of 1000−10 000 nm. This suggests that different mechanisms are involved in sol-to-gel transition of PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions as the temperature increases. The transition of unimer (3.5−4 nm)-to-polymer aggregation (1000−10 000 nm) is involved in the sol-to-gel transition of the PEG/PCL multiblock copolymer, whereas micelle (6 nm)-to-micellar aggregation (20 nm) is involved in the sol-to-gel transition of the PCL−PEG−PCL triblock copolymer. In a low-temperature sol phase of PEG/PCL multiblock copolymer, the system is not stiff enough to stop the mass flow of the polymer solution. As the temperature increases, the low molecular weight PEG blocks are partially dehydrated as suggested in ^{13}C NMR. Such a change can make the PEG/PCL multiblock copolymer more hydrophobic and increases the polymer−polymer attraction to form tight physical junctions. Water is trapped among the physical junctions, and the multiblock copolymer can form a gel.

Conclusions

Both PCL−PEG−PCL triblock and PEG/PCL multiblock copolymer aqueous solutions undergo a sol-to-gel transition as the temperature increases. However, the PCL−PEG−PCL triblock copolymer aqueous solution turned into an opaque gel in 1 h at room temperature. Raman spectroscopy, X-ray diffraction, optical microscopy, and differential scanning calorimetry suggest that crystallization of the PCL−PEG−PCL triblock copolymer in water is responsible for such phase behavior.

Like PCL−PEG−PCL triblock copolymer, PEG/PCL multiblock copolymer keeps the powder morphology, and their aqueous solution (20 wt %) shows maximal modulus at around body temperature (35−42 °C). The powder morphology of both polymers is clearly distinguished from previous thermogelling polymers in that it is easy to weigh, transfer, and dissolve the polymer in water. The PEG/PCL multiblock copolymer aqueous solution was stable as a transparent solution at room temperature and thus gives some practical convenience during the drug formulation.

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Supporting Information Available: SEM images of PCL−PEG−PCL triblock and PEG/PCL multiblock copolymers (Figure S1) and ^{13}C NMR spectra of PEG/PCL multiblock copolymer aqueous solution (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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