Thermogelling Aqueous Solutions of Alternating Multiblock Copolymers of Poly(L-lactic acid) and Poly(ethylene glycol)

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We are reporting alternating multiblock copolymers of poly(L-lactic acid)/poly(ethylene glycol) aqueous solution (>15 wt %) undergoing sol−gel−sol transition as the temperature increases from 20 to 60 °C. Micelles of the multiblock copolymers (in water) are about 20 nm in radius at low temperature. They are aggregated to a larger size as the temperature increases, which should play a critical role in the sol-to-gel transition. The transition temperature and gel window were affected by the molecular weight and composition of the multiblock copolymer. In particular, the aqueous solution of an alternating multiblock copolymer ($M_n \approx 6700$ daltons) prepared from poly(ethylene glycol) ($M_n \approx 600$ daltons) and poly(L-lactic acid) ($M_n \approx 1300$ daltons) showed a maximum modulus at body temperature (37 °C). The in situ gel forming ability of the polymer aqueous solution in vivo as well as in vitro indicates that it can be a promising injectable biomaterial.

Introduction

Reverse thermogelling polymers have been drawing attention for drug delivery and tissue engineering applications.[1,2] Typically, the aqueous solution is in a low viscous sol phase at room temperature or lower; however, it becomes a semisolid or gel as the temperature increases. Such a novel property enables pharmaceutical agents to be mixed in the sol (solution) state at room temperature, followed by injecting the solution into a target tissue to form a gel depot in situ. Poly(ethylene glycol/poly(lactic acid-*co*-glycolic acid),[3,4] chitosan derivatives,[5,6] poly(phosphazene)s,[7] poly(ethylene glycol)/poly(propylene fumarate),[8] and poly(ethylene glycol)/polycaprolactone[9] have been reported so far, as biodegradable reverse thermogelling polymers.

Polymers with a multiblock topology have been studied extensively as a biomaterial.[10,11] They were prepared by coupling the hydroxyl or carboxylic acid end groups using hexamethylene diisocyanate, phosgene, dicyclohexylcarbodiimide, or terephthaloyl chloride. Through the coupling reaction, the physicochemical properties could be easily controlled by varying the block length and total molecular weight of the polymer. Most of the biodegradable multiblock copolymers have been prepared from poly(lactide), polycaprolactone, poly(glycolic acid), poly(trimethylene carbonate), poly(hydroxybutyrate), and poly(ethylene glycol) to improve the physicochemical properties of the micro- and nanoparticle, and to improve the mechanical properties as biomaterials.[12−15] They are not soluble in water. Recently, the water-soluble multiblock copolymers consisting of poly(ethylene glycol) and poly(L-lactic acid) or polycaprolactone have been reported.[16,17] However, the aqueous solutions of poly(ethylene glycol)/poly(L-lactic acid) (PEG/PLLA) multiblock copolymers showed solution-to-precipitation, whereas those of poly(ethylene glycol)/polycaprolactone multiblock copolymers showed gel-to-sol transition (upper transition or gel melting) as the temperature increased.

Poloxamer, a triblock copolymer of poly(ethylene glycol) and poly(propylene glycol), was coupled by hexamethylene diisocyanate and terephthaloyl chloride to prepare the multiblock copolymers.[18,19] Alternatively, poly(ethylene glycol) and poly(propylene glycol) were coupled to prepared poly(ether carbonate).[20] Such an approach significantly prolongs the gel duration of poloxamer, which has been a main disadvantage of the poloxamer hydrogel for drug delivery and tissue engineering applications.

However, as far as we know, there is no report on the multiblock copolymer of biodegradable polyester showing reverse thermal gelation. We are reporting the poly(ethylene glycol)/poly(L-lactic acid) (PEG/PLLA) alternating multiblock copolymer showing reverse thermal gelation. Most of the polymers with a reverse thermal gelation were observed for the low molecular weight PEG as in the case of poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid), chitosan, poly(phosphazene)s, poly(propylene fumarate), and polycaprolactone.[3−9] Therefore, the molecular weight of PEG was fixed at 600 daltons in this research, and the total molecular weight of PEG/PLLA multiblock copolymer was varied to study the effect of molecular weight on the sol−gel transition of the aqueous polymer solution. In addition, the molecular weight of poly(L-lactic acid) (PLLA) was varied to see the effect of hydrophobicity of the PEG/PLLA multiblock copolymer on the sol−gel transition.

Materials and Methods

Materials. L-Lactide, stannous octoate, 1,6-hexane diol, poly(ethylene glycol) (MW = 600) (PEG), 1,6-diphenyl-1,3,5-hexatriene, methylene blue, succinic anhydride, succinic acid, dicyclohexylcarbodiimide, 4-*N*,*N*-dimethyl aminopyridine, tetrahydrofuran, and anhydrous toluene were used as received from Aldrich. Diethyl ether and *n*-hexane were used as received from Daejung (Korea).

PLLA Synthesis. The PLLA was prepared by typical ring-opening polymerization of L-lactide using stannous octoate as a catalyst.[21] 1,6-

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Table 1. List of PEG/PLLA Alternating Multiblock Copolymers

	feeding block	$R_{EG/LLA}$[a]	M_n[b]	M_w/M_n[b]
PI	PEG600/PLLA1100	1.1	4400	1.2
PII	PEG600/PLLA1300	0.9	4500	1.8
PIII	PEG600/PLLA1300	1.0	6700	2.2
PIV	PEG600/PLLA1300	1.1	7800	2.6
PV	PEG600/PLLA1500	0.9	5800	2.4

[a] The ratio of the number of ethylene glycol units to the number of lactic acid units of a multiblock copolymer ($R_{EG/LLA}$) was determined by the ^{1}H NMR (in $CDCl_3$) spectra. $R_{EG/LLA} = (0.25A_{3.6}/A_{5.2})$. $A_{3.6}$ and $A_{5.2}$ are the areas of the ethylene peak ($-CH_2CH_2O-$; 3.6 ppm) and the methine peak ($-CH(CH_3)COO-$; 5.2 ppm) of the PEG/PLLA multiblock, respectively. [b] Measured by gel permeation chromatography.

Hexane diol was used as an initiator. For example, to synthesize the PLLA used for the PEG/PLLA multiblock copolymer (PIII in Table 1), 1,6-hexane diol (0.98 g) was dissolved in anhydrous toluene (80 mL), and the solvent was distilled off to a final volume of 30 mL. L-Lactide (10.0 g) and stannous octoate (8 μL) were added to the reaction mixtures and stirred at 120 °C for 24 h. The product was isolated by precipitation into diethyl ether/*n*-hexane. The polymer was redissolved in 30 mL of methylene chloride and precipitated by slowly adding diethyl ether/*n*-hexane. The residual solvent was removed under vacuum.

^{1}H NMR ($CDCl_3$) of PLLA: δ 1.2−1.7 ($-OCH(CH_3)CO-$) and ($-OCH_2CH_2$ $CH_2CH_2CH_2O-$), δ 4.1 ($-OCH_2CH_2CH_2$ CH_2-CH_2CH_2O-; triplet), δ 4.3 ($HOCH(CH_3)CO-$; end group; quartet), δ 5.2 ($-OCH(CH_3)CO-$).

α,ω-Dicarboxylic Acid Terminated PLLA Synthesis. The succinic anhydride was reacted with PLLA to prepare α,ω-dicarboxylic acid terminated PLLA (PLLA-DA). For example, to prepare the PLLA-DA used for the PEG/PLLA multiblock copolymer (PIII in Table 1), PLLA (8.0 g), succinic anhydride (1.6 g), and succinic acid (0.04 g) were dissolved in toluene (80 mL). The reaction mixture was stirred at 100 °C for 6 h. The product was purified by precipitating into diethyl ether/*n*-hexane.

^{1}H NMR ($CDCl_3$) of α,ω-dicarboxylic acid terminated PLLA (PLLA-DA): δ 1.2−1.7 ($-OCH(CH_3)CO-$) and ($-OCH_2CH_2$ $CH_2CH_2CH_2O-$), δ 2.7 ($-OCCH_2CH_2CO-$), δ 4.1 ($-OCH_2CH_2CH_2$ $CH_2CH_2CH_2O-$; triplet), δ 5.2 ($-OCH(CH_3)CO-$).

PEG/PLLA Multiblock Copolymer Synthesis. The PEG/PLLA multiblock copolymer was prepared by the coupling reaction between PEG and α,ω-dicarboxylic acid terminated PLLA.[16] To control the molecular weight of the PEG/PLLA multiblock copolymer, a little excess amount of the PEG was used.[22] For example, to synthesize the PEG/PLLA multiblock copolymer (PIII in Table 1), PEG (3.00 g) was dissolved in anhydrous toluene (80 mL), and the solvent was distilled off to a final volume of 20 mL. Dried methylene chloride (50 mL) was added to the reaction mixture. α,ω-Dicarboxylic acid terminated PLLA (6.00 g), dicyclohexylcarbodiimide (2.38 g), and 4-*N*,*N*-dimethyl aminopyridine (0.21 g) were added to the reaction mixtures and stirred at room temperature for 24 h. The product was purified by precipitation into diethyl ether/*n*-hexane.

^{1}H NMR ($CDCl_3$) of PEG/PLLA multiblock copolymer: δ 1.2−1.7 ($-OCH(CH_3)CO-$) and ($-OCH_2CH_2$ $CH_2CH_2CH_2O-$), δ 2.7 ($-OCCH_2CH_2CO-$), δ 3.6 ($-OCH_2CH_2-$), δ 4.1 ($-OCH_2CH_2CH_2$ $CH_2CH_2CH_2O-$; triplet), δ 4.2 ($-OCH_2CH_2-OCOCH_2CH_2CO-$; triplet), δ 5.2 ($-OCH(CH_3)CO-$).

Gel Permeation Chromatography. The gel permeation chromatography (GPC) system (Waters 515) with a refractive index detector (Waters 410) was used to obtain the molecular weights and molecular weight distributions of the polymers. Tetrahydrofuran was used as an eluting solvent. The PEGs in a molecular weight range of 400−10 000 daltons were used as the molecular weight standards. Styragel HMW 6E and HR 4E columns (Waters) were used in series.

NMR Study. A 500 MHz NMR spectrometer (Varian) was used for ^{1}H NMR (in $CDCl_3$) to study the composition of the polymer and ^{13}C NMR to see the spectral change of the PEG/PLLA multiblock copolymer (PIII: 25 wt % in D_2O) as a function of temperature. The solution temperature was equilibrated for 20 min before the measurement.

In Situ Gel Formation. PEG/PLLA multiblock copolymer (PIII) aqueous solution (20 wt %; 0.3 mL) at room temperature was injected over 5 s into a vial containing 37 °C water. Blue dye (0.01 mg of methylene blue) was added to the polymer solution to see the gel clearly. The gel formation was photographed during the injection.

The in vivo gel formation was confirmed by subcutaneous injection of the PEG/PLLA multiblock copolymer (PIII) aqueous solution (20 wt %; 0.3 mL) into rats. Photographs were taken by surgery 48 h after the subcutaneous injection into the rat.

Sol−Gel Transition. The sol−gel transition was determined by the test tube inverting method.[23,24] The 4 mL vials (diameter 1.1 cm) containing 0.5 mL of PEG/PLLA multiblock copolymer aqueous solutions were immersed in a water bath at 10 °C for 20 min. 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. The transition temperature is an average of three measurements for each point.

The sol−gel transition of the PEG/PLLA multiblock copolymer aqueous solution was also investigated by dynamic mechanical analysis (Thermo Haake, Rheometer RS 1).[25,26] The aqueous polymer solution was placed between parallel plates of 25 mm diameter and a gap of 0.5 mm. 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.

Micelle Formation. Micelle formation was determined by the hydrophobic dye (1,6-diphenyl-1,3,5-hexatriene) solubilization method at room temperature.[27,28] The dye solution in methanol (10 μL at 0.4 mM) was injected into an aqueous polymer solution (1.0 mL) in a polymer concentration range of 0.0005−0.5 wt %. The absorption spectra (Shimadzu UV 2450) of these solutions were recorded from 300 to 400 nm. The absorbance at 378 nm relative to that at 400 nm was plotted against polymer concentration, and a crossing point of the two extrapolated straight lines was defined as the critical micelle concentration of the polymer.

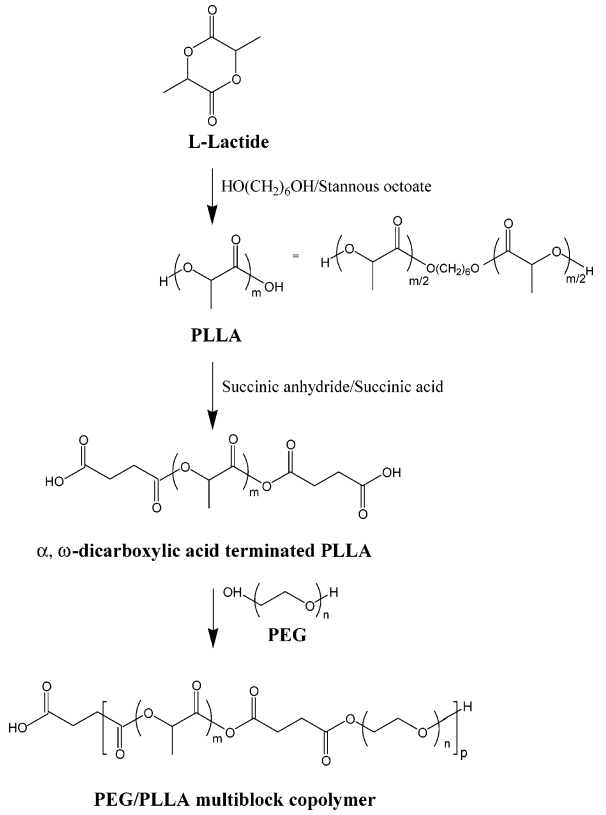
Dynamic Light Scattering. The size of a micelle was studied by a dynamic light scattering instrument (ALV 5000-60 × 0) as a function of temperature at the polymer concentrations of 0.1 and 1.0 wt % in deionized water. A YAG DPSS-200 laser (Langen, Germany) operating at 532 nm was used as a light source. Measurements of scattered light were made at an angle of 90° to the incident beam. The results were analyzed by the regularized CONTIN method. The decay rate distributions were transformed to an apparent diffusion coefficient. From the apparent diffusion coefficient, the hydrodynamic radius of a micelle can be obtained by the Stokes−Einstein equation.

Results and Discussion

The synthetic route of PEG/PLLA multiblock copolymer was described in Scheme 1. First, α,ω-dihydroxy terminated PLLA was prepared by ring-opening polymerization of L-lactide using 1,6-hexane diol. Second, the end groups of PLLA were modified to carboxylic acid by reacting an excess amount of succinic anhydride. Third, the dihydroxy end groups of PEG and dicarboxylic acid end groups of PLLA were coupled using dicyclohexylcarbodiimide to prepare the PEG/PLLA multiblock copolymer.

The initial molecular weight of PEG was 600 and that of PLLA was varied between 1100 and 1500. When the molecular weight of PLLA was smaller than 1100, the resulting PEG/PLLA mutiblock copolymer was soluble in water and did not give the sol-to-gel transition in a physiologically important temperature range of 30−45 °C. When the molecular weight of PLLA was larger than 1500, the resulting PEG/PLLA multiblock copolymer was not soluble in water. The PEG/PLLA

Scheme 1. Synthesis of PEG/PLLA Multiblock Copolymer



(600/1300) multiblock copolymer was not soluble when the molecular weight was larger than 7800. Therefore, the total molecular weight as well as the molecular weight of each block is an important molecular parameter to show sol-to-gel transition (reverse thermal gelation). Table 1 lists the PEG/PLLA multiblock copolymers studied in the research. The ratio of the number of ethylene glycol units to the number of L-lactic acid units of the PEG/PLLA multiblock copolymer ($R_{EG/LLA}$) is a measure of hydrophilicity of the polymer. The number of PEG blocks (x) and PLLA blocks (y) of a PEG/PLLA multiblock copolymer could be calculated by assuming that the block length of PEG and PLLA did not change during the coupling reaction and that the total molecular weight of the multiblock copolymer was given by the gel permeation chromatography. For PIII in Table 1,

$$A_{3.6}/A_{5.2} = \{(4 \times 13.6)x\}/\{(1 \times 18.1)y\}$$

$$600x + 1300y = 6700$$

$A_{3.6}$ and $A_{5.2}$ are the area of the ethylene peak ($-\boldsymbol{CH}_2CH_2O-$; 3.6 ppm) and the methine peak ($-\boldsymbol{CH}(CH_3)COO-$; 5.2 ppm) of the PEG/PLLA multiblock copolymer in the ^{1}H NMR (in $CDCl_3$) spectra, respectively. The values 13.6 and 18.1 are the number of repeating units of ethylene glycol in PEG 600 and lactic acid in PLLA 1300, respectively. Therefore, PIII can be described as a multiblock copolymer consisting of 4.2 PEG (MW = 600) blocks and 3.2 PLLA (MW = 1300) blocks in an alternating manner.

The progress of reaction could be traced by gel permeation chromatography (Figure 1). PEG (600 daltons), PLLA (1300 daltons), and PEG/PLLA multiblock copolymers (6700 daltons) (PIII) are shown at 18.6, 17.5, and 15.2 min, respectively. The change in ^{1}H NMR spectra around 4.1−4.3 ppm also shows the progress of the reaction (Figure 2). A triplet at 4.1 ppm

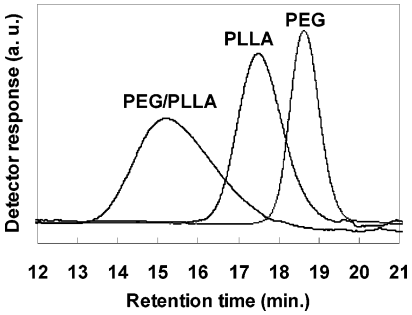


Figure 1. Gel permeation chromatogram of the PEG, PLLA, and PEG/PLLA multiblock copolymer (PIII).

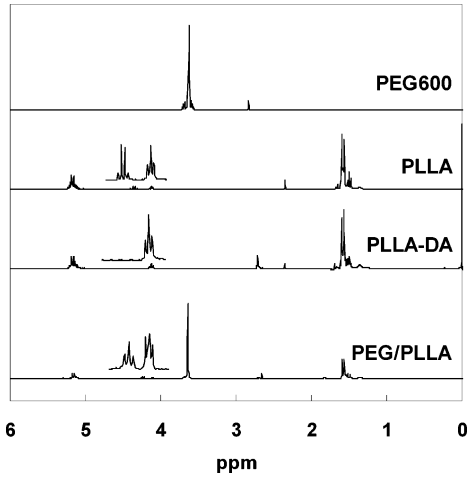


Figure 2. ^{1}H NMR spectra (in $CDCl_3$) of the PEG, PLLA, α,ω-dicarboxylic acid terminated PLLA (PLLA-DA), and PEG/PLLA multiblock copolymer (PIII).

comes from an initiator ($-O\boldsymbol{CH}_2CH_2CH_2\ CH_2CH_2\boldsymbol{CH}_2O-$), and a quartet at 4.3 ppm comes from the end group of PLLA ($HO\boldsymbol{CH}(CH_3)CO-$). The number average molecular weight (M_n) of the PLLA could be calculated by comparing these peaks with a methine peak of PLLA at 5.2 ppm. The quartet at 4.3 ppm disappeared in α,ω-dicarboxylic acid terminated PLLA. Because the hydroxyl end groups of PLLA reacted with succinic anhydride, only a triplet at 4.1 ppm appeared in the ^{1}H NMR spectra of α,ω-dicarboxylic acid terminated PLLA (PLLA-DA). In the PEG/PLLA multiblock copolymer, α,ω-dicarboxylic acid PLLA reacted with PEG. Two triplets of the PEG/PLLA multiblock copolymer at 4.1 and 4.2 ppm come from initiator ($-O\boldsymbol{CH}_2CH_2CH_2CH_2CH_2\boldsymbol{CH}_2O-$) and a connecting ethylene group of PEG to α,ω-dicarboxylic acid PLLA ($-OCH_2\boldsymbol{CH}_2$–$OCOCH_2CH_2CO-$), respectively.

When the vials containing polymer and water were rotated at 4 °C for 12 h, a transparent swollen polymer gel was obtained. When the swollen gel was kept at room temperature for 1 h, the gel was melted to a free-flowing polymer aqueous solution. The PEG/PLLA multiblock copolymer can be compared to PEG−PCL−PEG or PCL−PEG−PCL triblock copolymer in that the latter gave the aqueous polymer solution in a couple of minutes by a heating and quenching cycle whereas the former did not give the homogeneous polymer solution by the same procedure.[9,29] Once the PEG/PLLA multiblock copolymer is dissolved in water, the solution is a free-flowing sol at room temperature. Figure 3 shows the in situ gel formation from the polymer (PIII) aqueous solution (20 wt %). Injecting the polymer (PIII) aqueous solution (20 wt % at room temperature) using a syringe with a 21 gauge needle into water at 37 °C, the instantaneous gel formation was observed. To see the gel

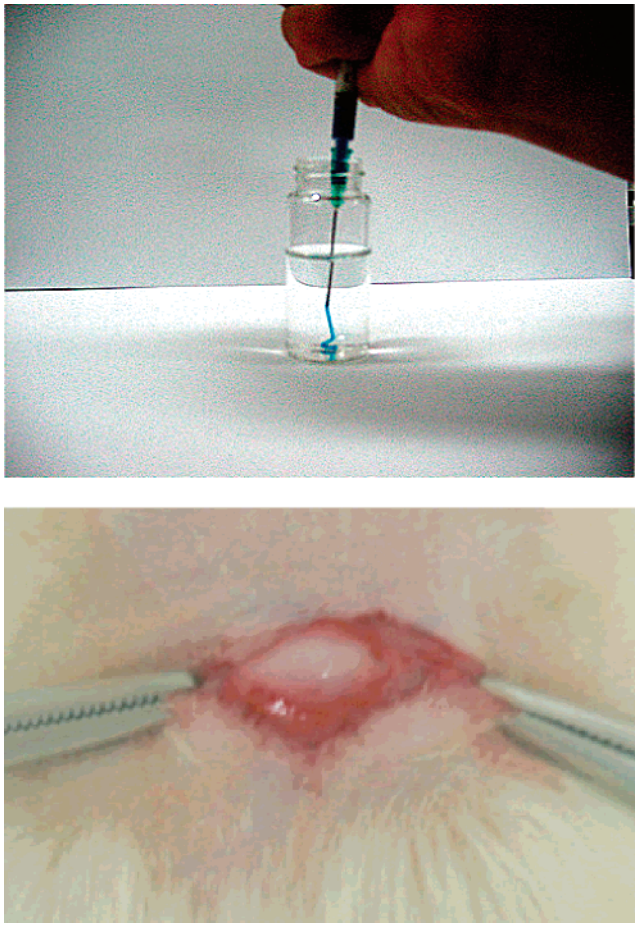


Figure 3. In situ gel formation of the PEG/PLA multiblock copolymers aqueous solution (PIII: 20 wt %). For in vitro study, the polymer aqueous solution at room temperature was injected into an excess amount of water at 37 °C (top). Methylene blue was added to see the gel clearly. The photograph of in vivo gel formation was also taken 48 h after the subcutaneous injection of PIII (20 wt %; 0.3 mL) to rats (bottom).

formation clearly, blue dye was added to the polymer solution. The aqueous polymer (PIII) solution (0.3 mL; 20 wt %) was also subcutaneously injected into rats using a syringe with a 21 gauge needle. Similar to other thermogelling polymers,[30,31] the in vivo gel formation was also confirmed by surgery 48 h after the injection of the PEG/PLLA multiblock copolymer aqueous solution.

The phase transition of the PEG/PLLA multiblock copolymer aqueous solution was studied by the test tube inverting method and dynamic mechanical analysis. The sol−gel transition accompanies an abrupt change in viscosity or modulus. Several methods have been developed to measure the transition temperature such as the test tube inverting method, the falling ball method, and the dynamic mechanical analysis. The transition temperatures determined by these methods coincided within 1−2 °C.[30] Figure 4 shows the phase diagram of the PEG/PLLA multiblock copolymer (PIII in Table 1) aqueous solution studied by the test tube inverting method based on flow (sol) or no-flow (gel) criterion.[23,24] At a low temperature, the polymer aqueous solution is in a transparent sol phase. As the temperature increases, it undergoes sol-to-gel transition between 30 and 37 °C depending on the polymer concentration. The gel phase turns into a turbid sol again at the higher temperature of 42−47 °C depending on the initial polymer concentration. The higher sol is a practically turbid suspension of the polymer in water. The turbid suspension formed by increasing the temperature of the

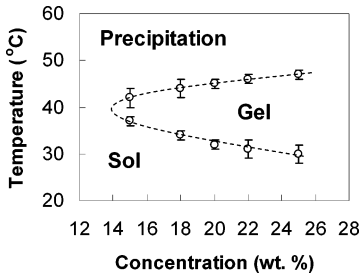


Figure 4. Phase diagram of PEG/PLLA multiblock copolymers (PIII) in deionized water. The temperature of the bath was changed in steps of 1 °C. Conditions of flow (sol) or no flow (gel) were determined by inverting a vial vertically in the bath.

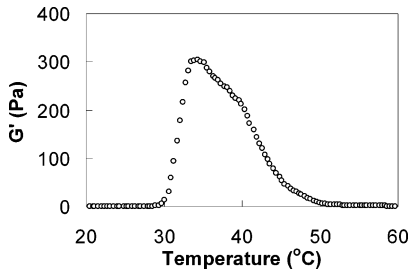


Figure 5. Dynamic mechanical analysis of the PEG/PLLA multiblock copolymers aqueous solution (PIII; 25 wt %) as a function of temperature. The heating rate was 0.5 °C/min.

polymer aqueous solution became a transparent sol by cooling to low temperature. As the temperature of the polymer solution (sol) increased again, the sol−gel transition occurred at the same temperature with the first heating cycle, indicating the reversibility of the sol−gel transition. Figure 5 shows the change in the storage modulus (G') of the 25 wt % aqueous polymer (PIII in Table 1) solution as the temperature increases with a heating rate of 0.5 °C/min. G' increased abruptly at the sol-to-gel transition of 30 °C and showed a maximal value at 33−35 °C, and then decreased to a low value enough to flow at 47 °C.

A polymer consisting of the hydrophilic PEG and hydrophobic PLLA blocks forms a core−shell structure in water. The hydrophilic PEG forms a shell, and the hydrophobic PLLA forms a core to minimize the free energy in water. The formation of the core−shell structure in water could be confirmed by partitioning of hydrophobic dye (1,6-diphenyl-1,3,5-hexatriene) in the presence of PEG/PLLA multiblock copolymer. At a fixed dye concentration, the absorbance at 337, 356, and 378 nm increased as the polymer concentration increased (Figure 6a). The partitioning of the hydrophobic dye to a hydrophobic core is responsible for such behavior.[27,28] The relative absorbance at 378−400 nm was used to determine the critical micelle concentration at 20 °C. The critical micelle concentration of the PEG/PLLA multiblock copolymer (PIII) was about 0.008 wt % (Figure 6b).

The micelle size was determined by dynamic light scattering (Figure 7). At a low concentration (0.1 wt %) of PEG/PLLA multiblock copolymer (PIII) in water, the average radius of a micelle was almost constant at 20−22 nm over 15−35 °C, and increased to 26 nm at 45 °C, and 32 nm at 55 °C. However, at a higher concentration (1.0 wt %), the average radius of a micelle increased from 21 nm at 15−25 °C to 31 nm at 35 °C. At higher temperatures of 45 and 55 °C, the broad distribution of the polymer size over 100−800 nm reflects the significant polymer aggregation. Considering that the sol−gel transition occurs above 15 wt %, the micelle aggregation contributes to the sol-to-gel transition of the PEG/PLLA multiblock copolymer

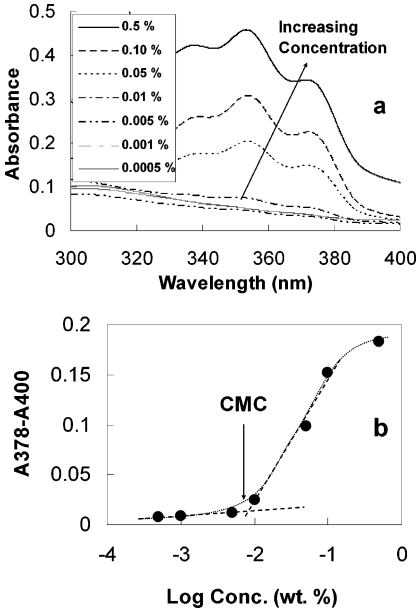


Figure 6. (a) UV−vis spectra of DPH as a function of PEG/PLLA multiblock copolymer (PIII) concentration (wt %) in water at room temperature. (b) Determination of the critical micelle concentration (CMC) by a crossing point of the two extrapolating lines at low and high concentration regions.

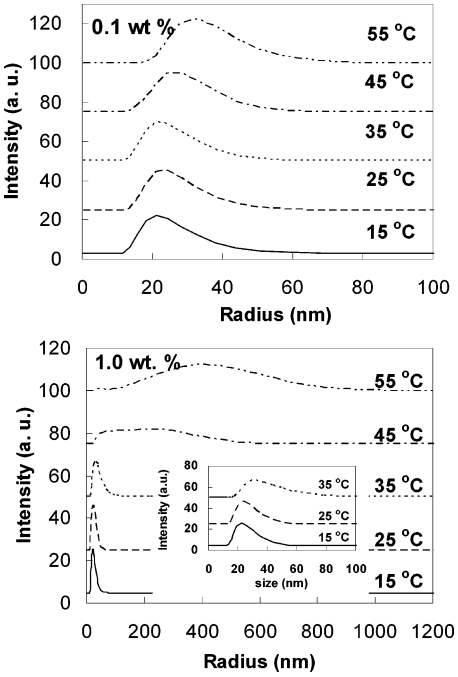


Figure 7. Size of the PEG/PLLA multiblock copolymer micelle in water (PIII: 0.1 wt % and 1.0 wt %) as a function of temperature obtained from dynamic light scattering.

aqueous solution and more significant polymer aggregation must be involved at gel-to-sol transition.

^{13}C NMR of PEG/PLLA multiblock copolymer (PIII) in D_2O (25 wt %) gave more information on the basis of the phase transition (Figure 8). As the temperature increased from 15 to 55 °C, the PEG peak at 69.7 ppm shifted to 70.3 ppm and broadened. A decrease in the molecular motion due to the dehydration of PEG was suggested to be responsible for such behavior.[3,9,32] On the contrary, the PLLA peak at 15 ppm just began to appear above 45 °C. The PLLA blocks are sequestered as a core of micelle structure in water. Thus, the PLLA peak is almost collapsed in the sol and gel phase. Above the gel-to-sol

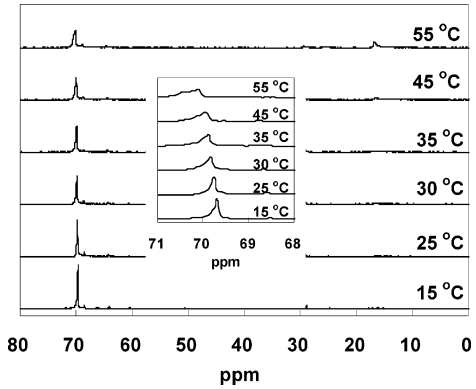


Figure 8. ^{13}C NMR spectra of PEG/PLLA multiblock copolymers (PIII) aqueous solution (25 wt %) as a function of temperature. The changes in the PEG peak (69−71 ppm) at 15 °C (lower sol phase), 25 °C (lower sol phase), and 30 °C (sol-to-gel transition temperature), and 35 °C (gel phase), 45 °C (gel phase), and 55 °C (upper sol phase) are inserted. The methyl peak of PLLA at 16.7 ppm is too small and appears just after the upper sol state (55 °C).

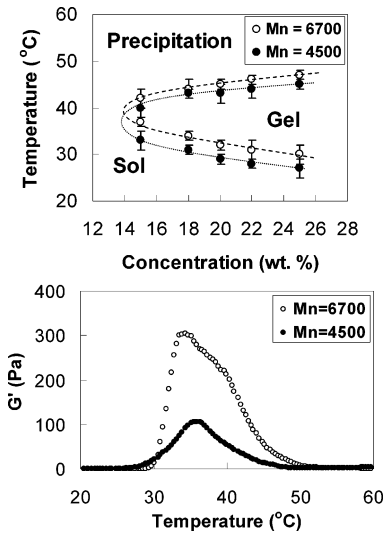


Figure 9. Effect of total molecular weight of PEG/PLLA multiblock copolymers (PII $M_n \approx 4500$) and PIII ($M_n \approx 6700$)) on the sol−gel transition multiblock copolymer aqueous solutions. PVI ($M_n \approx 7800$) was not soluble in water. The storage modulus (G') is shown as a function of temperature for the polymer aqueous solutions (25 wt %).

transition temperature (55 °C), the molecular motion of PLLA blocks increased and the PLLA peak appeared in ^{13}C NMR. Such an increase in the molecular motion of PLLA makes the system flow as a sol.

The control of sol−gel transition temperature, gel window, and critical gel concentration are primarily important parameters in designing an in situ gel forming system. Polymer solution is mixed with a drug or cells in a sol state around room temperature and is supposed to turn into a hydrogel depot at physiological environment (37 °C). Such parameters could be controlled by varying the molecular weight and composition of the PEG/PLLA multiblock copolymers. Figure 9 shows that the phase diagram is shifted 2−4 °C by increasing total molecular weight from 4500 to 6700. The high modulus of the multiblock copolymers (25 wt % in water) was observed at the similar temperature range of 35−40 °C. However, the modulus at 37 °C increased from 100 to 300 Pa by increasing the molecular weight of PEG/PLLA multiblock copolymer from 4500 to 6700 daltons. The higher molecular weight PEG/PLLA multiblock copolymer (PIV; $M_n \approx 7800$) was not soluble in water. As a thermogelling injectable biomaterial, the aqueous solution should be a low

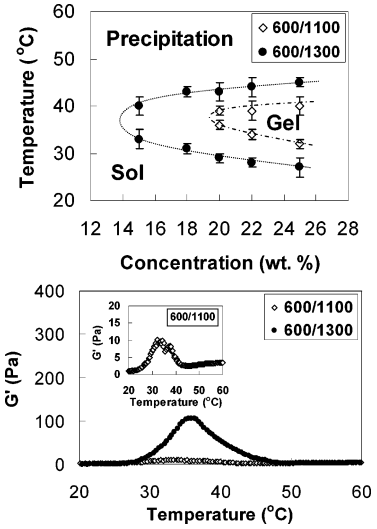


Figure 10. Effect of PLLA length of PEG/PLLA multiblock copolymers (PI (600/1100), PII (600/1300)) on the sol−gel transition of multiblock copolymer aqueous solutions. The storage modulus (*G*′) is shown as a function of temperature for the polymer aqueous solutions (25 wt %).

viscous sol at room temperature or lower, and form a semisolid gel at body temperature (37 °C). Therefore, the gel window of 30−45 °C and gel modulus (>100 Pa) at body temperature are important parameters. The PEG/PLLA multiblock copolymer (PIII) with a block length of 600/13 000 and total molecular weight of 6700 daltons showed promising properties for such a purpose.

By decreasing the PLLA molecular weight from 1300 to 1100 in PEG/PLLA multiblock copolymer, the sol-to-gel transition temperature increased by 5−7 °C. The critical gel concentration increased and gel window decreased as the PLLA molecular weight decreased. The decrease in hydrophobicity of the polymer makes the gel formation more difficult, and thus a higher temperature is needed for the polymer to aggregate as a hydrogel. The gel can be broken by a small increase in temperature. Therefore, the PEG/PLLA (600/1100) multiblock copolymer (PI) showed a smaller gel window than the PEG/PLLA (600/1300) multiblock copolymer (PII) (Figure 10). The maximum gel modulus of PEG/PLLA (600/1100) multiblock copolymer gel (25 wt %) was about 10 Pa. The PEG /PLLA (600/1500) multiblock copolymer with a higher molecular weight of PLLA was not soluble in water because the polymer was too hydrophobic. Therefore, the PLLA molecular weight should be controlled over 1100−1500 for the PEG (MW = 600)/PLLA multiblock copolymer to show reverse thermal gelation in a physiologically important temperature range of 30−45 °C.

Conclusions

The alternating PEG/PLLA multiblock copolymer showing reverse thermal gelation was developed by coupling PEG and PLLA. At a fixed PEG molecular weight of 600, the PLLA should be in a range of 1100−1500 daltons for the PEG/PLLA multiblock copolymer to show a reverse thermal gelation in a physiologically important temperature range of 30−45 °C. The micelle aggregation seems to be the gelation mechanism as supported by dye solubilization, dynamic light scattering, and ^{13}C NMR. The transition temperature can be controlled by varying the molecular weight and composition of the PEG/PLLA multiblock copolymer. In particular, the PEG/PLLA (600/1300) with total molecular weight of 6700 showed itself to be promising as a biodegradable injectable system as confirmed in vivo and in vitro in situ gel forming experiments.

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