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Article

Size-Controlled Nanomicelles of Poly(lactic acid)–Poly(ethylene glycol) Copolymers with a Multiblock Configuration

Shota Somekawa [1,2], Kazunari Masutani [2], Yu-I Hsu [2], Atsushi Mahara [1], Yoshiharu Kimura [2] and Tetsuji Yamaoka [1,*]

[1] Department of Biomedical Engineering, National Cerebral and Cardiovascular Center Research Institute Fujishiro-dai, Suita, Osaka 565-8565, Japan; E-Mails: d3861002@edu.kit.ac.jp (S.S.); mahara@ncvc.go.jp (A.M.)

[2] Department of Biobased Materials Science, Kyoto Institute of Technology Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan; E-Mails: kzn16@hotmail.com (K.M.); cathy860313@hotmail.com (Y.-IH.); ykimura@kit.ac.jp (Y.K.)

* Author to whom correspondence should be addressed; E-Mail: yamtet@ncvc.go.jp; Tel.: +81-6-6833-5012 (ext. 2637); Fax: +81-6-6835-5476.

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Abstract: The ability to control the micelle size of poly(lactic acid) and poly(ethylene glycol) (PLA–PEG) block copolymers is important for controlling their circulation in blood cell recognition, drug release and therapeutic effects. We successfully controlled the micelle size by changing the block number of copolymers (multiblock index). PLA–PEG multiblock copolymers with multiblock indexes ranging from 1.35 to 2.78 were synthesized by direct polycondensation with tin chloride/*p*-toluenesulfonic acid binary catalysts, using PEG with a molecular weight (M_w) of 3200 Da. The M_w of PLA–PEG copolymers increased with an increase in the multiblock index, while micelle size, measured by dynamic light scattering, decreased greatly from 349 to 28 nm. In addition, the X-ray diffraction peak of the PLA crystal disappeared when the multiblock index was increased. These results indicate that a multiblock structure is useful for controlling micelle size without changing the PLA/PEG composition or PEG molecular weight, which strongly influences other micelle features.

Keywords: micelle size; multiblock copolymer; poly(lactic acid); poly(ethylene glycol)

1. Introduction

Diblock and triblock copolymers of poly(lactic acid) and poly(ethylene glycol) (PLA–PEG copolymers) possess excellent biocompatibility and biodegradability. It is well known that these block copolymers form micelle structures in water, with a PLA core and PEG shell, owing to the amphiphilicity of hydrophilic PEG and hydrophobic PLA. These structures have been investigated for use as drug carriers [1], along with other amphiphilic block copolymers [2]. We previously reported a novel function of the micelle aqueous suspension. When heated, the mixed suspension of poly(L-lactic acid)–PEG micelles and poly(D-lactic acid)–PEG micelles forms a hydrogel based on the rearrangement of the micelle structure to form a network structure with stereocomplex crosslinking points, which can be used as an injectable hydrogel-type biomaterial [3,4].

The topologies of the PLA–PEG copolymer micelles have been extensively studied. PLA–PEG–PLA triblock copolymers form flower-like micelles with the PEG blocks [3–5], while PLA–PEG diblock copolymers tend to form star-like micelles [4,5]. These micelles can enclose hydrophobic drugs in the core and are highly stable in the blood for a long period of time. Since micelle size affects various functions and efficacies, methods for size control are very important and have been studied extensively. In general, the micelle size of PLA/PEG block copolymers is controlled by the PLA/PEG composition and total molecular weight. Yue *et al*. reported *in vivo* behavior of PEG–*b*–PLA micelles with a size of 30 to 150 nm. They also controlled the micelle size by changing the PLA block length. The amount of micelles that accumulated in each organ depended on the micelle size, and the smaller micelles were more effective in tumor-growth inhibition [6].

However, it is not easy to change PLA–PEG copolymer characteristics in a wide range. Generally, the molecular weight of PEG used for block copolymers is less than 50,000 Da, because higher molecular weight PEGs are not excreted from the kidney and may accumulate in the body [7]. If the molecular weight of PEG in PLA–PEG diblock or PLA–PEG–PLA triblock copolymers is fixed, the PLA/PEG composition determines not only the amphiphilicity, but also the total molecular weight of the copolymers.

To solve these problems, the development of a multiblock structure is one of the most promising strategies. In 1999, we developed multiblock copolymers composed of PEG and water-soluble polyether segments [8]. We confirmed that tissue encapsulation of these multiblock copolymers was reduced, along with inflammatory cytokine production [9]. The multiblock structure enabled independent control of the segment length, total molecular weight and compositions of block copolymers. Multiblock copolymers with high molecular weight can even be processed to form films and fibers, while copolymers with high and low PLA content are effective as sutures or adhesion prevention membranes [10,11]. The multiblock copolymers also form nanoparticles in the water. In 2000, Bae *et al*. and Na *et al*. reported nanoparticles of PLA–PEG and poly(ε-caprolactone)–PEG (PCL–PEG) multiblock copolymers [12,13].

In the present study, the effect of the multiblock index (expressed as "*n*" in $(PLA–PEG)_n$) of PLA–PEG multiblock copolymers on their micelle size was investigated. PLA–PEG multiblock copolymers with different multiblock indexes were synthesized by changing the reaction temperature and reaction time in direct polycondensation, using tin (II) chloride dihydrate ($SnCl_2$) and *p*-toluenesulfonic acid monohydrate (TSA) as a binary catalyst system. This binary catalyst system

does not require a chain extender and was developed in our group to synthesize higher molecular weight PLLA (poly(L-lactide)) with low racemization and less coloring [14–16].

2. Experimental Section

2.1. Materials

The D-lactic acid (LA) aqueous solution (90%) was purchased from Musashino Chemical Laboratory, Ltd. (Tokyo, Japan). PEG with a weight average molecular weight (M_w) of 3200 Da, succinic acid (SA) and TSA were purchased from Nacalai Tesque Inc. (Kyoto, Japan). $SnCl_2$ was purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Polycondensation

LA (0.25 mol), PEG (7.0 mmol) and SA (6.8 mmol) were charged into a three-necked flask equipped with a mechanical stirrer and a reflux condenser. The temperature of the reflux condenser was maintained at 100 °C using a ribbon heater. At first, the air in the flask was replaced with argon gas. The flask was immersed in an oil bath at 150 °C, and the pressure was reduced to 66.7 kPa, with the conditions maintained for 5 h. Since SA might be removed from the system at high temperature and reduced pressure, SA was allowed to react with LA and PEG at 150 °C and 66.7 kPa. Then, $SnCl_2$ (1 wt% relative to the total amount of LA and PEG) and TSA (an equimolar amount to $SnCl_2$) were added into the flask, and the flask was heated to 150 or 180 °C. The pressure was reduced gradually to reach 0.67 kPa and maintained for 15, 30 and 60 h at 150 °C or 10, 20, 30 and 60 h at 180 °C. The products were dissolved in dichloromethane, and the resulting solutions were poured into an excess amount of cold diethyl ether. The precipitated products were filtered and dried under vacuum for 24 h.

2.3. Preparation of Micelle Suspension

Ten milligrams of copolymer were dissolved in 100 μL of tetrahydrofuran (THF), and the solution was dropped into 1 mL of distilled water. THF was removed from the solution by stirring at room temperature overnight. The final concentration of the micelle suspension was adjusted to 1 and 7 wt% by changing the volume of distilled water.

2.4. Measurement

^{1}H NMR spectra were recorded on a Bruker ARX-600 spectrometer (Bruker Co., Karlsruhe, Germany) at 600 MHz with deuterated chloroform ($CDCl_3$) containing 0.03 vol% tetramethylsilane (TMS) used as an internal standard. The number average molecular weight (M_n), M_w and molecular weight distribution (M_w/M_n) were determined by gel permeation chromatography (GPC). The analyzer was composed of a Shimadzu LC-10AD pump and a RID-10A refractive-index detector (Shimadzu Co., Kyoto, Japan). A set of Tosoh TSK gel-GMH_{HR}-H columns (particle size (mean): 5 μm. 7.8 mm I.D. × 30 cm) was used with $CHCl_3$ as the eluent at a flow rate of 0.3 mL/min at 35 °C. The molecular weights were calibrated with polystyrene standards. We termed the number of repeating units (n) of

$(PLA–PEG)_n$ as the multiblock index. Copolymers with n = 1 and 1.5 represent diblock and triblock copolymers, respectively. The multiblock index of PLA–PEG copolymers was calculated from the PLA/PEG block ratio (NMR) and M_w (GPC).

The light transmittance of a micelle suspension of 1 wt% was measured using a spectrometer on a Shimadzu UV-1800 at a wavelength of 500 nm at room temperature. The micelle size was determined by dynamic light scattering (DLS) on a nano ZS Zetasizer at 20 °C (Malvern Instruments Ltd., Worcestershire, UK).

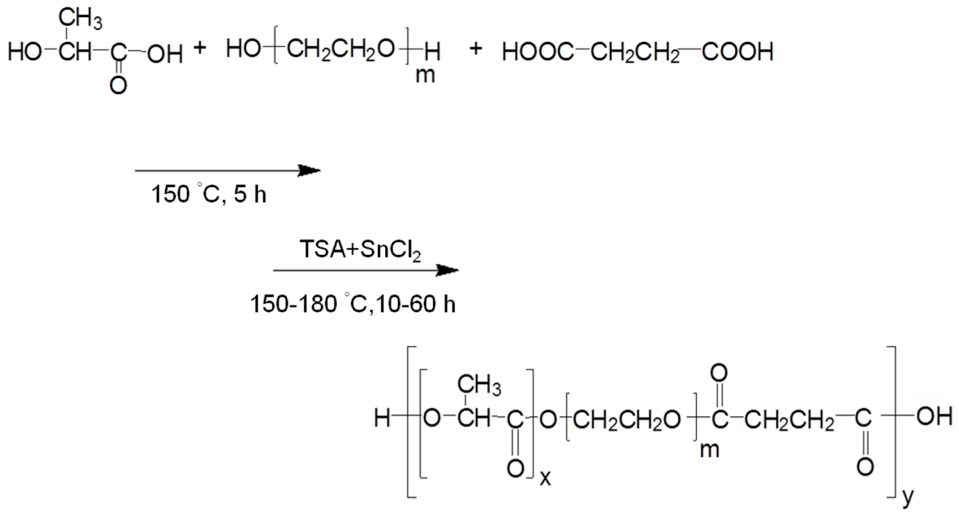
The micelle morphology was observed by atomic force microscopy (AFM) on multimode equipment (Veeco Co, Ltd., Plainview, NY, USA). Five microliters of micelle suspension (1 wt%) were dropped on the mica plate, dried at room temperature and observed in tapping mode using a silicon cantilever with a force constant of 40 N/m and a typical resonant frequency of 325 kHz.

Wide angle X-ray diffraction (WAXD) of micelles was measured on an RINT 2100 FSL refractometer (Rigaku Co., Tokyo, Japan). The PLA–PEG multiblock micelle suspension (7 wt%) was freeze-dried and measured. The scattering intensities were scanned in the 2θ range of 5 to 40° at a scan rate of 2°/min at room temperature. The relative PLA crystal peak intensity was calculated by evaluating the area under the WAXD diffraction peaks (PLA crystal/(total crystal + total amorphous)).

3. Results

3.1. Synthesis of Multiblock Copolymers

Multiblock copolymers consisting of PLA, PEG and SA were synthesized by direct polycondensation using binary catalysts of $SnCl_2$ and TSA (Scheme 1). The reaction was confirmed by ^{1}H-NMR. As shown in Figure 1, methine and methyl protons in PLA appeared at around 5.2 ppm and 1.5 ppm, respectively, and the signals of main chain methylene units in the PEG blocks appeared at 3.7 ppm. The α-methylene protons of the ethylene glycol units connected to PLA appeared at 4.3 ppm, together with the methine protons of the hydroxylated lactyl end units. The proton peaks of SA (δ = 2.7 ppm) were observed from the resultant polymer by ^{1}H-NMR. This result coincides with several earlier studies of PLA–PEG multiblock copolymers [17–19].



Scheme 1. Synthesis of the poly(lactic acid) (PLA)–PEG multiblock copolymer.

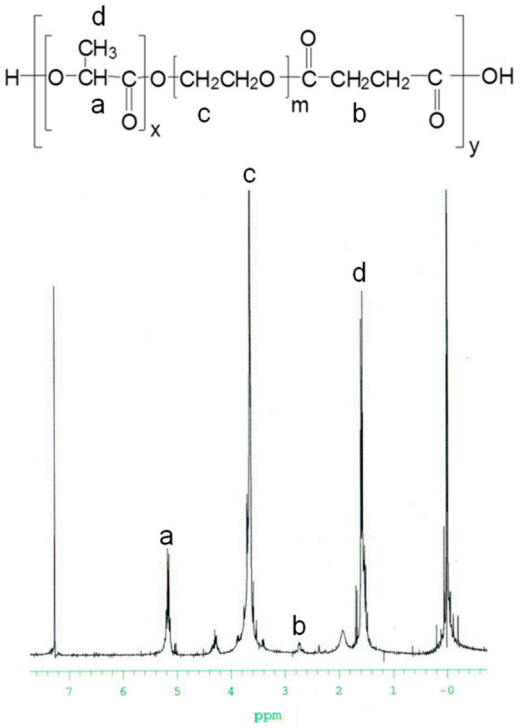


Figure 1. [1]H-NMR spectra of the PLA–PEG multiblock copolymer in $CDCl_3$.

The characteristics of the copolymers are summarized in Table 1. The LE(m)-*x* indicates a PLA–PEG (LE) multiblock copolymer (m) with the multiblock index of *x*. Figure 2 shows the changes in the M_w of PLA–PEG copolymers as a function of reaction time at 150 and 180 °C. Before the catalysts were added, the M_w and M_n of the polymer were 3700 and 3400 Da, respectively. At 150 °C, the M_w gradually increased with increasing reaction time to reach 9200 Da after 65 h. On the other hand, at 180 °C, the M_w sharply increased to 14,000 Da after 25 h, but gradually decreased afterwards. There was an obvious decrease in M_w at 65 h. Long-term heating and high reaction temperatures might induce the decomposition of the PLA segments in the PLA–PEG copolymer.

Table 1. Characteristics of the PLA–PEG copolymers.

Sample name	Temperature (°C)	Reaction time (h)	M_n [a] (Da)	M_w [a] (Da)	M_w/M_n [a]	PLA/PEG [b] (*w*/*w*)	Multiblock index [c]
LE(m)-1.35	150	20	3,600	6,100	1.67	29.1:70.9	1.35
LE(m)-1.53	150	35	4,200	6,900	1.66	29.1:70.9	1.53
LE(m)-2.27	150	65	6,900	9,200	1.34	21.2:78.8	2.27
LE(m)-1.38	180	15	4,300	7,000	1.62	37.1:62.9	1.38
LE(m)-2.78	180	25	9,600	14,000	1.46	36.4:63.6	2.78
LE(m)-2.75	180	35	8,300	13,400	1.63	34.3:65.7	2.75
LE(m)-2.93	180	65	9,100	12,700	1.40	26.2:73.8	2.93

[a] Determined by GPC (flow rate: 0.3 mL/min; eluent: $CDCl_3$; molecular weight standard: polystyrene); [b] calculated from [1]H-NMR; [c] calculated from [1]H-NMR and GPC.

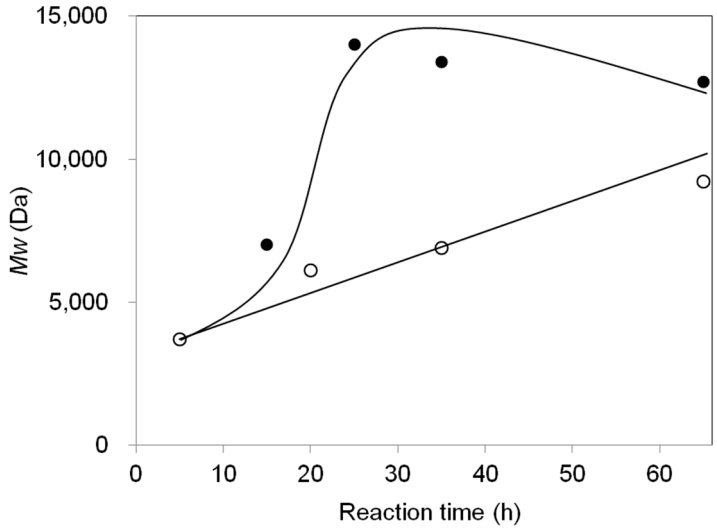


Figure 2. Changes in the molecular weight of the PLA–PEG copolymers as a function of the reaction time at 150 (○) and 180 °C (●).

Figure 3 shows typical GPC curves of the reprecipitated PLA–PEG multiblock copolymers. A bimodal peak was observed at 35 h when the reaction temperature was 180 °C. The small peak was probably due to a small amount of LA oligomers. The PLA of the PLA–PEG multiblock copolymer end units might have decomposed by transesterification. On the other hand, the bimodal peak was not observed at 5, 15, 25 or 65 h. The LA oligomers might have decomposed by 65 h and probably disappeared during reprecipitation. The PLA/PEG ratio measured by ^{1}H-NMR spectroscopy supports this result. The PLA content of the multiblock copolymer decreased with an increase in reaction time at 180 °C.

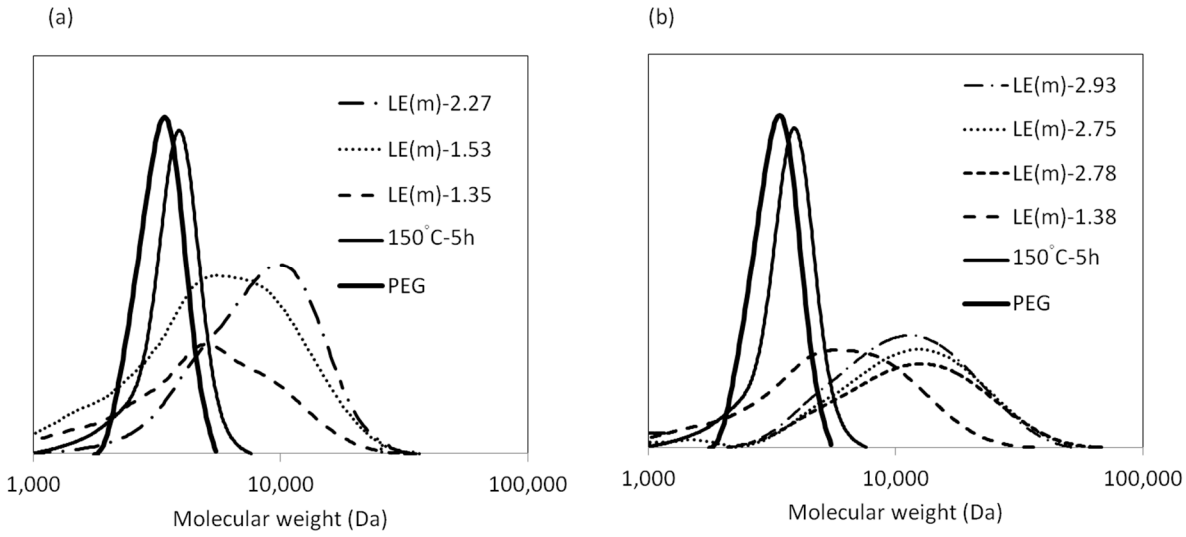


Figure 3. GPC curve of multiblock copolymers at (**a**) 150 and (**b**) 180 °C for each reaction time.

The PLA content of all of the PLA–PEG copolymers was similar to the feed ratio, but changed slightly according to the reaction conditions. The PLA content of the PLA–PEG multiblock copolymer was 29.1% when it was synthesized at 150 °C for 20 and 35 h. At 150 °C for 65 h, it was 21.2%. When the polymerization was conducted at 180 °C for 15, 25 and 35 h, it was about 36%. There was an

obvious decrease in the PLA content (26.2%) when the reaction time was 65 h. The PLA content of the product synthesized at the high reaction temperature was higher than that of the product synthesized at the low reaction temperature. The multiblock index, which was calculated by the unit ratio of PLA/PEG (NMR) and M_w (GPC), increased with an increase in reaction temperature and time. At 150 °C, the multiblock index increased with time and reached 2.27 at 65 h. At 180 °C, the multiblock index was 2.78 at 25 h and reached 2.93 after 65 h. Figure 4 shows the methyl protons decoupled from the methine protons of the PLA–PEG multiblock copolymers. The spectral signals were assigned according to previous studies [20–23]. The subscripts i and s indicate isotactic and syndiotactic, respectively. It was difficult to quantify the racemization from the NMR spectrum, but we tried to calculate the racemization ratio ((isi + sss + iis/ssi + sis)/(iii + ssi/iss + isi + sss + iis/ssi + sis)) and show the result in Figure S1. At 150 °C, the racemization ratio slowly increased with time, while at 180 °C, it rapidly increased after 35 h and reached over 30% at 65 h. In addition, the LE(m)-2.93 changed color to dark brown at 65 h of polymerization at 180 °C. Since these results indicate the possibility of racemization, we omitted the results of LE(m)-2.93 from the entire analysis.

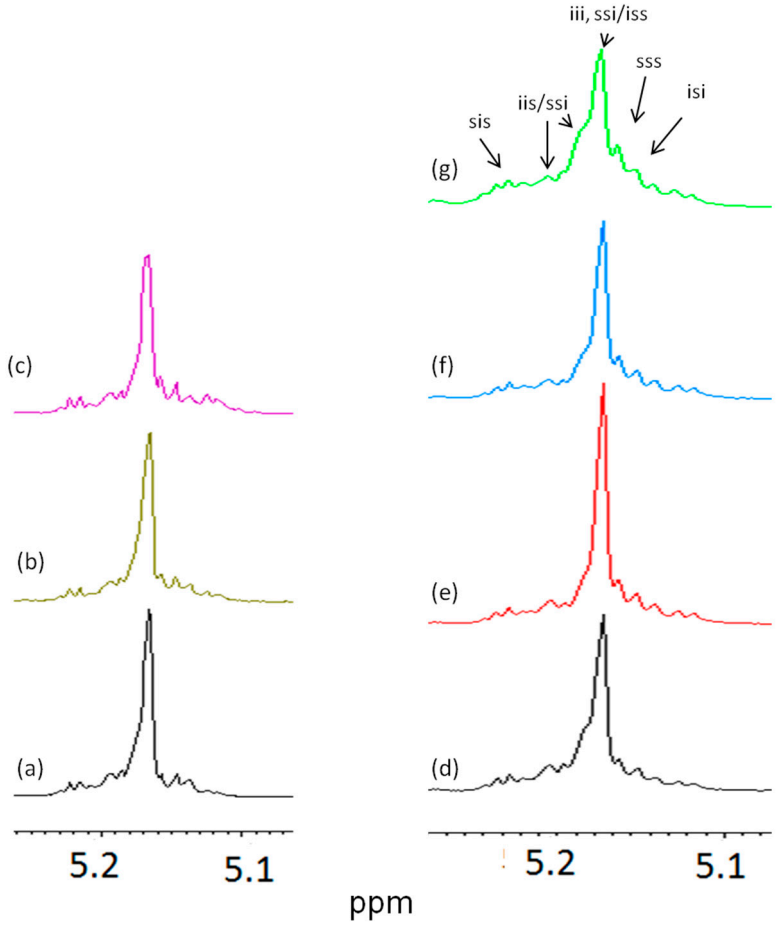


Figure 4. Methine proton signals from the homonuclear-decouple ^{1}H-NMR spectra of (a) LE(m)-1.35, (b) LE(m)-1.53, (c) LE(m)-2.27, (d) LE(m)-1.38, (e) LE(m)-2.78, (f) LE(m)-2.75 and (g) LE(m)-2.93 (i, isotactic; s, syndiotactic).

3.2. Micelles

Figure 5a shows the effect of the multiblock index on the light transmittance of the PLA–PEG multiblock copolymer micelle suspensions (1 wt%) at room temperature. Light transmittance of LE(m)-1.35, LE(m)-1.38 and LE(m)-1.53 was less than 35%, while that of LE(m)-2.27, LE(m)-2.75 and LE(m)-2.78 was higher than 80%. The correlation coefficient (r) between the multiblock index and the light transmittance was $r = 0.96$ ($p = 0.002$). In contrast, no correlation was observed between light transmittance and the PLA content of copolymers at room temperature (Figure 5b). The correlation coefficient was $r = -0.13$ ($p = ns$). Figure 6a shows the dependence of the particle size of the PLA–PEG multiblock copolymer micelle, in 1 wt% suspension at 20 °C, on the multiblock index. Interestingly, micelle size clearly decreases with the increased multiblock index, although the *Mw* of the copolymer increases with the increased multiblock index, as shown in Table 1. Figure S2 clearly shows that the particle size decreased with the increasing *Mw* of the block copolymers, which does not generally occur. The particle size of LE(m)-1.35, LE(m)-1.38, LE(m)-1.53, LE(m)-2.27, LE(m)-2.75 and LE(m)-2.78 was 349, 209, 218, 74, 28 and 38 nm, respectively. Their correlation coefficient was $r = -0.94$ ($p = 0.006$). The smaller size at the higher multiblock index is in good agreement with the higher light transmittance (Figure 5a). It is clear that the PLA contents is not a determinant of the particle size (Figure 6b, $r = -0.10$ ($p = ns$)).

The AFM image revealed that all of the PLA–PEG multiblock copolymers micelles were spherical (Figure 7). The majority of micelles of LE(m)-2.27, LE(m)-2.75 and LE(m)-2.78 were small at a diameter of 30 to 100 nm, while all of the micelles of LE(m)-1.35, LE(m)-1.38 and LE(m)-1.53 were much larger. These results agree with the DLS measurement.

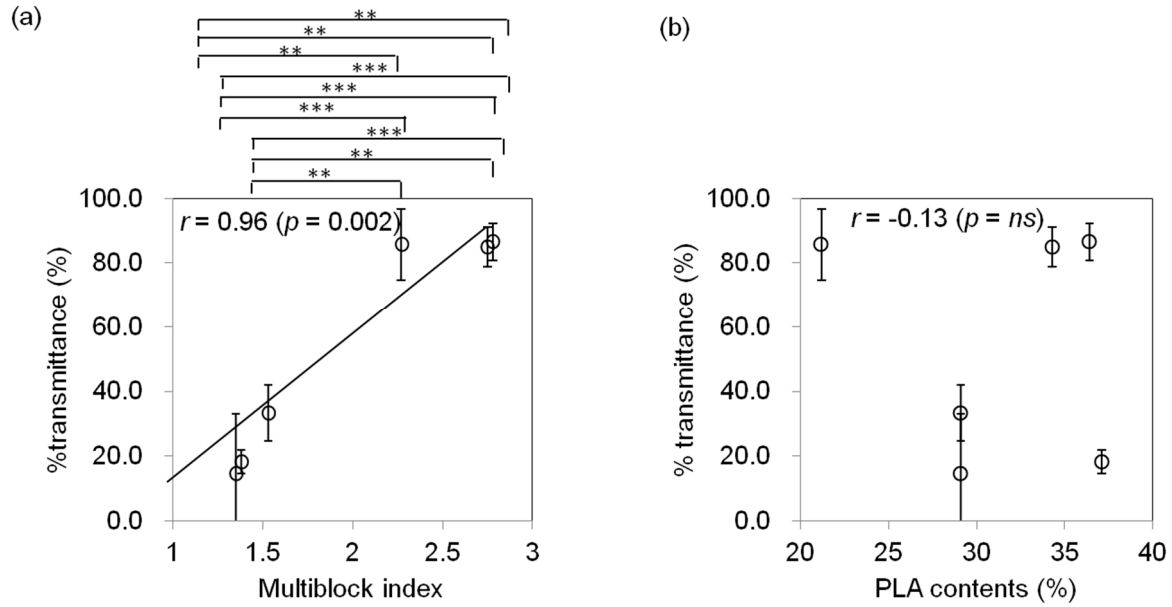


Figure 5. Light transmittance change of the PLA–PEG copolymers suspension (1 wt%) (**a**) multiblock indexes and (**b**) PLA content at room temperature (** $p < 0.005$, *** $p < 0.0001$).

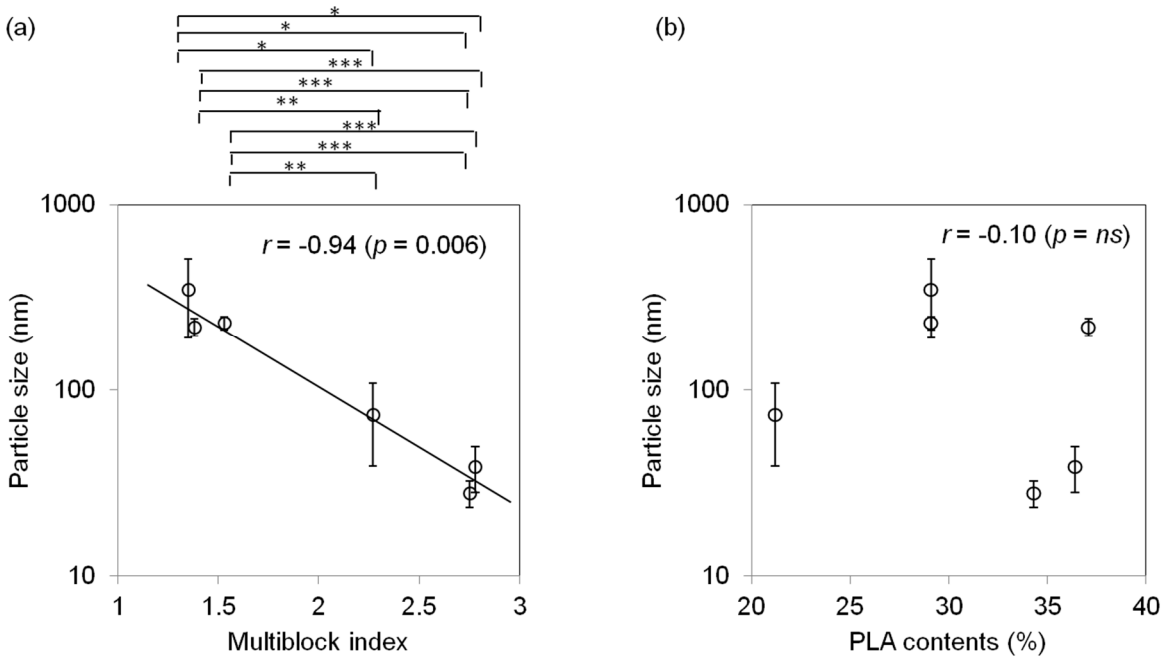


Figure 6. Change in the micelle suspension (1 wt%) of PLA–PEG copolymers as a function of (**a**) multiblock indexes and (**b**) PLA contents at 20 °C (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$).

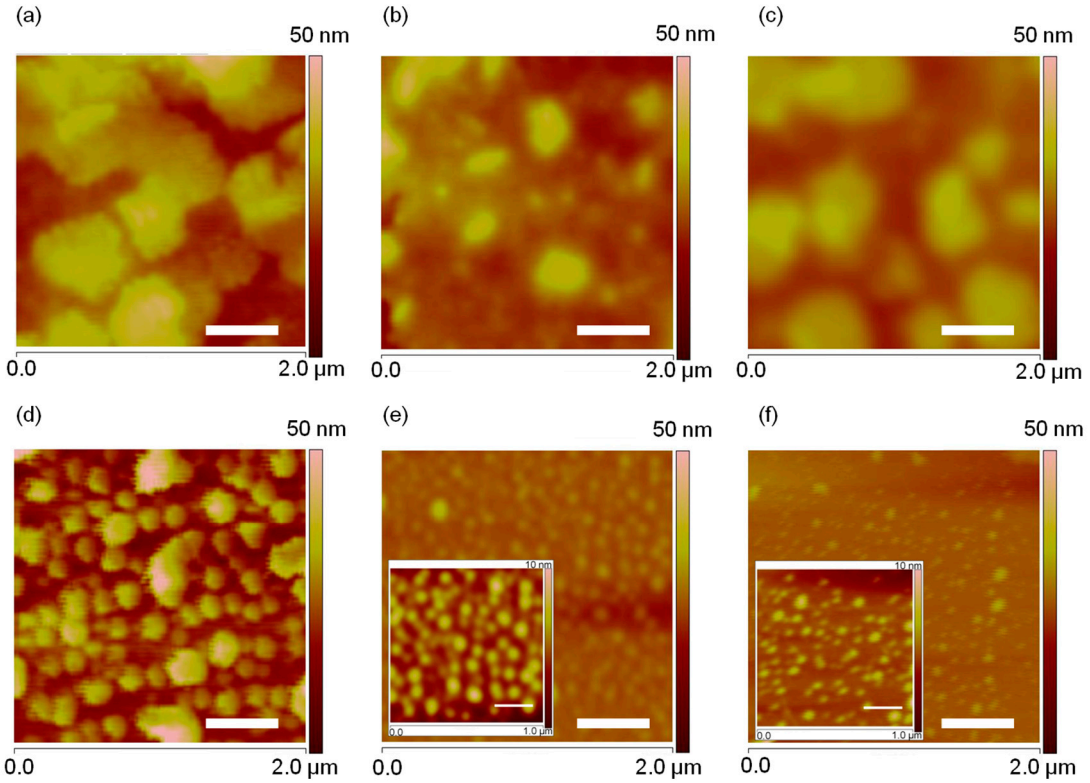


Figure 7. AFM images of the (**a**) LE(m)-1.35, (**b**) LE(m)-1.38, (**c**) LE(m)-1.53, (**d**) LE(m)-2.27, (**e**) LE(m)-2.75 and (**f**) LE(m)-2.78 micelles. The AFM image size and height are 2 µm × 2 µm and 50 nm, respectively (scale bar = 500 nm). The image size and height of the enlargement images are 1 µm × 1 µm and 10 nm, respectively (scale bar = 250 nm).

Figure 8 shows the WAXD profiles of the micelles. The PEG crystal peaks are located at 2θ = 23.5°. The peaks at 2θ = 19° are the overlapping peaks of PLA and PEG. LE(m)-1.35, LE(m)-1.38 and LE(m)-1.53 micelles show PLA crystal peaks at 2θ = 17°, while LE(m)-2.27, LE(m)-2.75 and LE(m)-2.78 micelles do not. The relative PLA crystal peak intensity was calculated by PLA crystal (diffraction peaks of 17°)/(total crystal + total amorphous). Figure 9 indicates a clear decrease in the relative PLA crystal peak intensity with an increase in the multiblock index.

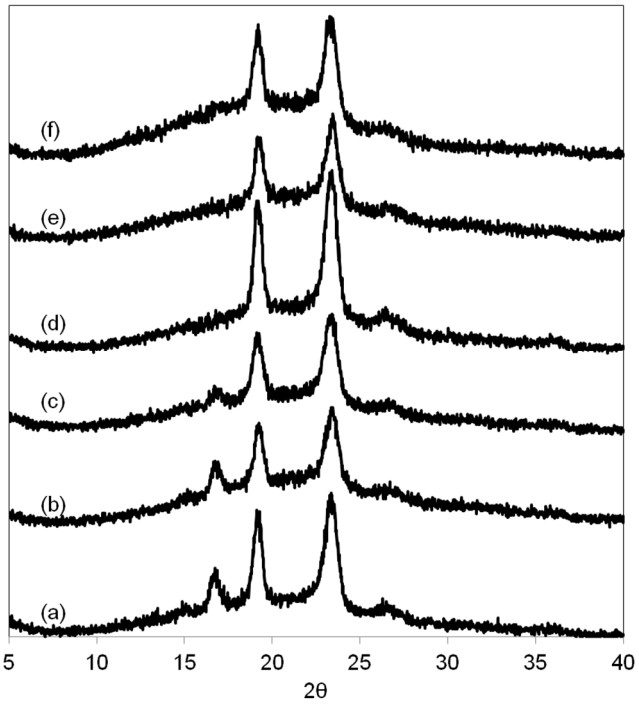


Figure 8. WAXD profile of the (a) LE(m)-1.35, (b) LE(m)-1.38, (c) LE(m)-1.53, (d) LE(m)-2.27, (e) LE(m)-2.75 and (f) LE(m)-2.78 micelles.

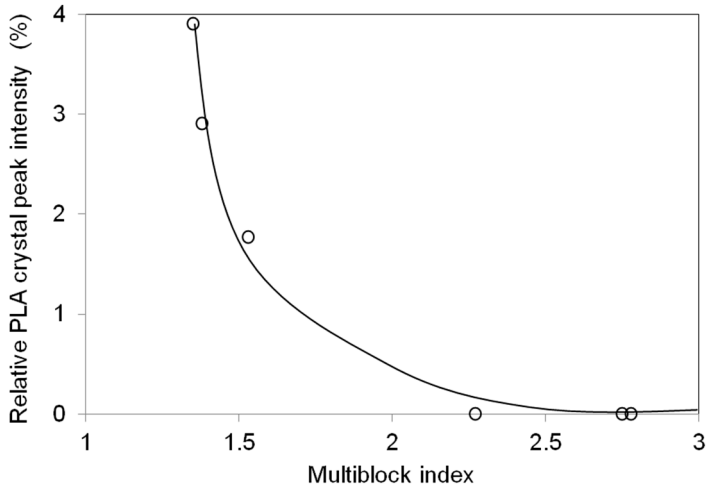


Figure 9. Relative PLA crystal peak intensity in the micelle of PLA–PEG multiblock copolymers as a function of multiblock indexes.

4. Discussions

4.1. Racemization

Our group reported that high molecular weight PLLA (about 100,000 Da) can be synthesized directly from LA without discoloring or racemization, through the use of binary catalysts [14–16]. Baick *et al*. reported the reversible oligomerization of L-lactic acid in this binary catalyst system [24]. Miao *et al*. applied this binary catalyst method to the synthesis of PLA–PEG–PLA triblock copolymers [25]. The reaction time, temperature, pressure and ratio of binary catalysts were studied to determine their effect on polymer decomposition, discoloring and racemization [14–16,24,25]. We previously reported that a reaction time longer than 20 h in melt polycondensation of PLLA was ineffective for further polymerization, but rather induced discoloration and racemization of PLLA [14]. In the present study, we succeeded in preparing PLA–PEG multiblock copolymers using binary catalysts without chain extenders for the first time. However, we observed obvious racemization and decomposition during polymerization at 180 °C for 65 h (Figure 4). We then excluded this data point from the main analysis.

4.2. Micelle Size

It is generally known that the micelle size of PLA–PEG block copolymers depends on the balance of hydrophobic and hydrophilic components (PLA and PEG ratio), as well as the PLA and PEG block lengths [4,5,13,26–28]. However, in our results, PLA contents ranging from 21.2% to 37.1%, which affect the hydrophilic-hydrophobic balance, were not a determinant of the micelle size. The micelle size depended rather on the multiblock index with an inverse correlation. A large multiblock index resulted in a small micelle diameter, although an increasing multiblock index increases the copolymer molecular weight (Table 1). It is hard to conclude that the multiblock index is the dominant parameter, but we could show that it must be one of the effective parameters for determining the micelle diameter.

Recently, Hadjiantoniouet *et al*. prepared and evaluated non-biodegradable multiblock copolymers from hydrophobic 2-(dimethylamino) ethyl methacrylate and hydrophilic methyl methacrylate, bearing from two to six blocks. Variations in the number of blocks led to differences in micelle size, micelle structure and aggregation number [29]. We also tried to evaluate the micelle structures.

4.3. Crystallinity

It is known that the crystallinity of both the PEG and the PLA segments decreases in the copolymer, and the crystallinity of multiblock copolymers is lower than that of triblock copolymers. The crystallinity also changes with block length and molecular weight. Lee *et al*. suggested that the presence of succinic acid might have inhibited PLA crystallization. Moreover, the crystallinity of PLLA and PEG in PLLA–PEG multiblock copolymers was influenced by both the molecular weight and the PLLA/PEG ratio [30].

In this study, we assessed the crystallinity of the PLA segment in the micelle structures. The multiblock copolymer with low PLA content (LE(m)-1.35) clearly showed the PLA crystal peak, but the multiblock

copolymer with large PLA content (LE(m)-2.78) did not. The multiblock index affected PLA crystal formation of PLA–PEG copolymers to a greater extent than PLA content did (Figure 9).

Glavas *et al*. reported that the crystallinity of the micelle core influenced micelle size and critical micelle concentration [31]. Micelles with a semicrystalline core (PEG–PCL) and those with an amorphous core (PEG–PεDL) showed different trends in size as a function of the hydrophobic/hydrophobic ratio. Semicrystalline micelles were smaller than amorphous micelles when the hydrophobic ratio was high (the hydrophobic/hydrophilic ratio was 2.0). On the other hand, semicrystalline micelles were larger than amorphous micelles when the hydrophobic ratio was low (the hydrophobic/hydrophilic ratio was 0.5). The micelle core crystallinity might be an important factor that influences micelle size.

Agrawal *et al*. reported that the crystallinity of the PLA core greatly affects the release of incorporated drugs [32]. They compared the micelles of poly(DL-lactic acid)–DLLA–PEG–poly(DL-lactic acid) (amorphous core) with the micelles of PLLA–PEG–PLLA (crystal core). The crystalline PLA core released drugs from the micelle at a relatively fast rate, whereas the amorphous PLA core showed a slower release. They suggested that the reason for the difference was the packing of PLA chains in the core. Moreover, the report suggested that the aggregation number of crystal core micelles was larger than that of amorphous core micelles [33]. The core crystallinity, which can be altered by the multiblock index, may also be an important factor in determining the micelle size and drug releasing behavior.

5. Conclusions

In this study, we synthesized PLA–PEG multiblock copolymers by a simple direct polycondensation, using $SnCl_2$ dihydrate and TSA as binary catalysts. The ^{1}H-NMR and GPC analysis showed that the multiblock index can be controlled by controlling the reaction time and temperature. It was found that the multiblock index of the biodegradable PLA–PEG copolymer is one of the major factors influencing the micelle size in aqueous solutions, as well as the crystallinity of the PLA core. The effect of these features on drug incorporation properties, drug releasing behavior and thermoresponsive gelation capacity will be investigated in the future.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/2073-4360/7/6/1177/s1.

Acknowledgments

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Author Contributions

Shota Somekawa conducted the entire experimental work under the supervision of Tetsuji Yamaoka (Corresponding author), Yoshiharu Kimura, Kazunari Masutani, and Atsushi Mahara. Yu-I Hsu assisted the WAXD experiment. The Manuscript was finally written by Shota Somekawa and Tetsuji Yamaoka.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Gref, R.; Minamitake, Y.; Peracchia, M.T.; Trubetskoy, V.; Torchilin, V.; Langer, R. Biodegradable long-circulating polymeric nanospheres. *Science* **1995**, *263*, 1600–1603.
2. Kataoka, K.; Harada, A.; Nagasaki, Y. Block copolymer micelles for drug delivery: Design, characterization and biological significance. *Adv. Drug Deliv. Rev.* **2001**, *47*, 113–131.
3. Fujiwara, T.; Mukose, T.; Yamaoka, T.; Yamane, H.; Sakurai, S.; Kimura, Y. Novel thermo-responsive formation of a hydrogel by stereo-complexation between PLLA–PEG–PLLA and PDLA–PEG–PDLA block copolymers. *Macromol. Biosci.* **2001**, *1*, 204–208.
4. Fujiwara, T.; Miyamoto, M.; Kimura, Y.; Iwata, T.; Doi, Y. Self-organization of diblock and triblock copolymers of poly(L-lactide) and poly(oxyethylene) into nanostructured bands and their network system. Proposition of a doubly twisted chain conformation of poly(L-lactide). *Macromolecules* **2001**, *34*, 4043–4050.
5. Fujiwara, T.; Kimura, Y. Macromolecular organization of poly(L-lactide)-*block*-polyoxyethylene into bio-inspired nano-architectures. *Macromol. Biosci.* **2002**, *2*, 11–23.
6. Yue, J.; Liu, S.; Xie, Z.; Xing, Y.; Jing, X. Size-dependent biodistribution and antitumor efficacy of polymer micelle drug delivery systems. *J. Mater. Chem. B* **2013**, *1*, 4273–4280.
7. Yamaoka, T.; Tabata, Y.; Ikada, Y. Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. *J. Pharm. Sci.* **1994**, *83*, 601–606.
8. Yamaoka, T.; Takahashi, Y.; Ohta, T.; Miyamoto, M.; Murakami, A.; Kimura, Y. Synthesis and properties of multiblock copolymers consisting of poly(L-lactic acid) and poly(oxypropylene-co-oxyethylene) prepared by direct polycondensation. *J. Polym. Sci. A Polym. Chem.* **1999**, *37*, 1513–1521.
9. Ehashi, T.; Kakinoki, S.; Yamaoka, T. Water absorbing and quick degradable PLLA/PEG multiblock copolymers reduce the encapsulation and inflammatory cytokine production. *J. Artif. Organs* **2014**, *17*, 321–328.
10. Yamaoka, T.; Takahashi, Y.; Fujisato, T.; Kimura, Y. Preparation and evaluations of PLLA-based multiblock copolymer fibers as a novel suture. *Jpn. J. Artif. Organs* **2000**, *29*, 239–244.
11. Yamaoka, T.; Njatawidjaja, E.; Kasai, A.; Agudelo, C.A.; Ehashi, T.; Kakinoki, S.; Kato, S.; Mahara, A. Elastic/adhesive double-layered PLA–PEG multiblock copolymer membranes for postoperative adhesion prevention. *Polym. Degrad. Stabil.* **2013**, *98*, 2168–2176.

12. Bae, Y.H.; Huh, K.M.; Kim, Y.; Park, K.H. Biodegradable amphiphilic multiblock copolymers and their implications for biomedical applications. *J. Control. Release* **2000**, *64*, 3–13.
13. Na, K.; Lee, K.H.; Lee, D.H.; Bae, Y.H. Biodegradable thermo-sensitive nanoparticles from poly(L-lactic acid)/poly(ethylene glycol) alternating multi-block copolymer for potential anti-cancer drug carrier. *Eur. J. Pharm. Sci.* **2006**, *27*, 115–122.
14. Moon, S.I.; Lee, C.W.; Miyamoto, M.; Kimura, Y. Melt polycondensation of L-lactic acid with Sn(II) catalysts activated by various proton acids: A direct manufacturing route to high molecular weight poly(L-lactic acid). *J. Polym. Sci. A Polym. Chem.* **2000**, *38*, 1673–1679.
15. Moon, S.I.; Taniguchi, I.; Miyamoto, M.; Kimura, Y.; Lee, C.W. Synthesis and properties of high-molecular-weight poly(L-lactic acid) by melt/solid polycondensation under different reaction conditions. *High Perform Polym.* **2001**, *13*, S189–S196.
16. Moon, S.I.; Deguchi, K.; Miyamoto, M.; Kimura, Y. Synthesis of polyglactin by melt/solid polycondensation of glycolic/L-lactic acids. *Polym. Int.* **2004**, *53*, 254–258.
17. Huh, K.M.; Bae, Y.H. Synthesis and characterization of poly(ethylene glycol)/poly(L-lactic acid) alternating multiblock copolymers. *Polymer* **1999**, *40*, 6147–6155.
18. Luo, W.; Li, S.; Bei, J.; Wang, S. Dependence of morphology on composition of poly(L-lactide)–poly(ethylene glycol) multiblock copolymers. *Polym. Adv. Technol.* **2002**, *13*, 233–238.
19. Chen, W.; Luo, W.; Wang, S.; Bei, J. Synthesis and properties of poly(L-lactide)–poly(ethylene glycol) multiblock copolymers by coupling triblock copolymers. *Polym. Adv. Technol.* **2003**, *14*, 245–253.
20. Fukushima, K.; Kimura, Y. An efficient solid-state polycondensation method for synthesizing stereocomplexed poly(lactic acid)s with high molecular weight. *J. Polym. Sci. A Polym. Chem.* **2008**, *46*, 3714–3722.
21. Kricheldorf, H.R.; Boettcher, C.; Tonnes, K.U. Polylactones: 23. Polymerization of racemic and meso D,L-lactide with various organotin catalysts-stereochemical aspects. *Polymer* **1992**, *33*, 2817–2824.
22. Thakur, K.A.M.; Kean, R.T.; Hall, E.S.; Kolstad, J.J.; Lindgren, T.A. High-resolution ^{13}C and ^{1}H solution NMR study of poly(lactide). *Macromolecules* **1997**, *30*, 2422–2428.
23. Ovitt, T.M.; Coates, G.W. Stereochemistry of lactide polymerization with chiral catalysts: New opportunities for stereocontrol using polymer exchange mechanisms. *J. Am. Chem. Soc.* **2002**, *124*, 1316–1326.
24. Baick, I.H.; Luciani, C.V.; Park, S.Y.; Lim, T.; Choi, K.Y. Kinetics of reversible oligomerization of L-lactic acid with a $SnCl_2 \cdot 2H_2O$/*p*-toluenesulfonic acid catalyst. *Ind. Eng. Chem. Res.* **2012**, *51*, 16617–16625.
25. Miao, P.; Zhao, C.; Xu, G.; Fu, Q.; Tang, W.; Zeng, K.; Wang, Y.; Zhou, H.; Yang, G. Degradation of poly(D,L-lactic acid)-*b*-poly(ethylene glycol)-*b*-poly(D,L-lactic acid) copolymer by electron beam radiation. *J. Appl. Polym. Sci.* **2009**, *112*, 2981–2987.
26. Wu, X.; Ghzaoui, A.E.; Li, S. Anisotropic self-assembling micelles prepared by the direct dissolution of PLA/PEG block copolymers with a high PEG fraction. *Langmuir* **2011**, *27*, 8000–8008.

27. Jie, P.; Venkatraman, S.S.; Min, F.; Freddy, B.Y.; Huat, G.L. Micelle-like nanoparticles of star-branched PEO–PLA copolymers as chemotherapeutic carrier. *J. Control. Release* **2005**, *110*, 20–33.
28. Zhao, H.; Liu, Z.; Park, S.; Kim, S.H.; Kim, J.H.; Piao, L. Preparation and characterization of PEG/PLA multiblock and triblock copolymer. *Bull. Korean Chem. Soc.* **2012**, *33*, 1638–1642.
29. Hadjiantoniou, N.A.; Triftaridou, A.I.; Kafouris, D.; Gradzielski, M.; Patrickios, C.S. Synthesis and characterization of amphiphilic multiblock copolymers: Effect of the number of blocks on micellization. *Macromolecules* **2009**, *42*, 5492–5498.
30. Lee, S.Y.; Chin, I.J.; Jung, J.S. Crystallization behavior of poly(L-lactide)–poly(ethylene glycol) multiblock copolymers. *Eur. Polym. J.* **1999**, *35*, 2147–2153.
31. Glavas, L.; Olsen, P.; Odelius, K.; Albertsson, A.C. Achieving micelle control through core crystallinity. *Biomacromolecules* **2013**, *14*, 4150–4156.
32. Agrawal, S.K.; Sanabria-Delong, N.; Coburn, J.M.; Tew, G.N.; Bhatia, S.R. Novel drug release profiles from micellar solutions of PLA–PEO–PLA triblock copolymers. *J. Control. Release* **2006**, *112*, 64–71.
33. Agrawal, S.K.; Sanabria-Delong, N.; Tew, G.N.; Bhatia, S.R. Structural characterization of PLA–PEO–PLA solutions and hydrogels: Crystalline *vs.* amorphous PLA domains. *Macromolecules* **2008**, *41*, 1774–1784.