Enhanced Crystallization of Poly(L-lactide-*co*-ε-caprolactone) in the Presence of Water

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ABSTRACT: Poly(L-lactide-*co*-ε-caprolactone) [P(LLA-CL)], which is used in biodegradable biomedical materials such as drug-delivery systems, surgical sutures, orthopedics, and scaffolds for tissue engineering, has been reported to crystallize upon storage in a dry state even at room temperature; this results in rapid changes in the mechanical properties. In biomedical applications, P(LLA-CL) is used in the presence of water. This study investigated the effects of water on the crystallization of P(LLA-CL) at 37°C in phosphate buffered solution, which was anticipated to alter its mechanical properties and hydrolytic degradation behavior. Surprisingly, the crystallinity of P(LLA-CL) in the presence of water rapidly increased in 6–12 h and then slowly increased up to 120 h. The period of time for the initial rapid crystallization increase in the presence of water was much shorter than that in the absence of water. The obtained information would be useful for the selection, preparation, and use of P(LLA-CL) in various biomedical applications. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 715–720, 2009

Key words: biodegradable; biomaterials; crystallization; structure–property relations

INTRODUCTION

The use of biomaterials in clinical applications has increased dramatically in the past few decades. As biomaterials, poly(L-lactide) [i.e. poly(L-lactic acid)] (PLLA) and poly(ε-caprolactone) (PCL) have attracted much interest because they have hydrolyzability, very low toxicity to the human body, and high mechanical performance and, thereby, can assist with tissue regeneration.[1–14] Biodegradable materials should have appropriate mechanical performance, degradation behavior, and controlled drug-release properties, depending on their applications and purposes. PLLA is glassy at room temperature (glass-transition temperature ≅ 60°C), whereas PCL is rubbery at room temperature (glass-transition temperature ≅ −60°C). The copolymerization of lactide (LA) with ε-caprolactone (CL) is a representative method for the control of the mechanical properties, degradation rate, and controlled drug-release properties, which thereby widens the range of their possible biomedical applications, such as in surgical sutures, wound closure, prosthetic implants, controlled-release systems, and tissue engineering scaffolds.[15–23]

PLLA, PCL, and their copolymers degrade via random-chain cleavage at ester groups autocatalyzed by the carboxylic acid end groups. The morphology and, particularly, the degree of crystallinity will influence the hydrolytic degradation kinetics in clinical applications. In phosphate buffered solution, crystallized PLLA specimens degraded more rapidly than amorphous ones, in marked contrast with the results in enzyme solution or alkaline solution. The decrease in the mechanical properties, molecular weight, and remaining weight of PLLA after hydrolytic degradation became higher with the initial crystallinity. On the other hand, when PLLA films were hydrolytically degraded in enzyme solution or alkaline solution, the weight loss rate was lower for PLLA films having a higher initial crystallinity.[14,24,25]

Degradation-induced changes in highly ordered structures, such as crystallization, are now considered a major phenomenon in hydrolytic degradation. Tsuji et al.[26] observed the enhanced crystallization of poly(L-lactide-*co*-ε-caprolactone) [P(LLA-CL)(50/50)] during storage in the absence of water even at room

temperature. Seppälä and coworkers[27,28] studied the hydrolytic degradation of CL copolymers with LAs (CL/L-LA or DL-LA = 80/20, 60/40, and 40/60 with both L-LA and DL-LA) and monitored the changes in the crystallinity and mechanical properties during hydrolytic degradation. Li et al.[29] and Tsuji et al.[30] observed that amorphous-made PLLA crystallized in aqueous media during hydrolytic degradation. Not only amorphous-made PLLA but also P(LLA-GA) crystallized in aqueous media as a result of hydrolytic degradation,[31] whereas P(LLA-CL) crystallized even in the absence of aqueous media, that is, during storage in a dry state.[26]

In a previous study, we investigated the effects of rapid crystallization on the hydrolytic degradation behavior of amorphous-made P(LLA-CL) in phosphate buffered solution.[32] This study disclosed the following results: The increase in the crystallinity of films hydrolytically degraded in phosphate buffered solution and stored in a dry state occurred rapidly in the first 14 days and, after that, slowed down. Furthermore, the crystallization of P(LLA-CL) induced dramatic changes in the mechanical properties.[26,32] In terms of the mechanical matching of a material to a tissue in biomedical applications, such mechanical property changes in P(LLA-CL) during storage or use can cause trouble. Despite this issue, as far as we are aware, there has been no report on the crystallization of P(LLA-CL) in the presence and absence of water in periods of time shorter than 14 days.

The objectives of this study were to investigate the rapid crystallization of P(LLA-CL) in the presence of water, as in hydrolytic degradation, and in the absence of water, as in storage, and to compare the results obtained in the presence and absence of water. For these purposes, the crystallization behavior of P(LLA-CL) in the presence and absence of water was investigated at 37°C in short periods of time up to 5 days. The crystallization of P(LLA-CL) was monitored by X-ray diffractometry and polarization optical microscopy.

EXPERIMENTAL

Materials

P(LLA-CL) was supplied kindly by Gunze, Ltd. (Kyoto, Japan) and purified by precipitation with methylene chloride and methanol as the solvent and nonsolvent, respectively, followed by drying *in vacuo* for 7 days. The films of the purified polymer (ca. 100 and 25 μm thickness) were prepared by the casting of 1.0 g/dL solutions, with methylene chloride as the solvent.[30,33] The solvent was allowed to evaporate at room temperature (25°C) for approximately 2 days; this was followed by drying *in vacuo* for 7 days.[28,31] As-cast films were made amorphous before the crys-

tallization experiments by the following procedure. Each of the films was placed between two Teflon sheets and sealed in a glass tube under reduced pressure. The sealed films were melted at 200°C for 3 min and quenched at 0°C.[34,35] During quenching, the specimen reached 0°C at least in 1 min.

Crystallization

For the crystallization in the presence of water, each of the amorphous-made films (3 × 30 × 100 mm^3 or 25 μm) was immersed in 10 mL of 0.15M phosphate buffered solution (pH = 7.4 ± 0.1) containing 2.0 mg of sodium azide (Nacalai Tesque, Inc., Kyoto, Japan, guaranteed grade) at 37°C for predetermined periods of time. The distilled water used for the preparation of phosphate buffered solution was high-performance liquid chromatography grade (Nacalai Tesque). For the crystallization of the film in the absence of water, each of the amorphous-made films (18 × 30 × 100 mm^3 or 25 μm) was sealed in a storage bag at 37°C for predetermined periods of time. The films crystallized in the presence and absence of water are abbreviated as P(LLA-CL)-H and P(LLA-CL)-S, respectively.

Measurements

The X-ray diffractometry of the films (thickness ≈ 100 μm) was carried out at 25°C with a Rigaku RINT-2500 (Tokyo, Japan) equipped with a Cu-Kα source (λ = 0.154 nm). The morphology of the films (thickness ≈ 25 μm) was studied with an Olympus BX50 polarization optical microscope (Tokyo, Japan). For the measurement of tensile properties, the P(LLA-CL)-H film was washed thoroughly with distilled water at room temperature; this was followed by drying under reduced pressure for 2 weeks. The tensile properties of films were measured at 25°C and 50% relative humidity with a Shimadzu EZ-Test tensile tester (Kyoto, Japan) at a crosshead speed of 100%/min. The initial gauge length of the specimen was always kept at 20 mm. The characteristics and properties of the melt-quenched P(LLA-CL) films before crystallization are tabulated in Table I.

RESULTS AND DISCUSSION

X-ray diffractometry

Figures 1 and 2 show the X-ray diffraction profiles of the P(LLA-CL)-H and P(LLA-CL)-S films, respectively, at different crystallization times. Here, the crystallization time is defined as the period of time for the films in phosphate buffered solution or in a storage bag. The initial P(LLA-CL)-H and P(LLA-CL)-S films before crystallization were completely

TABLE I
Characteristics and Properties of the Melt-Quenched P(LLA-CL) Film Before Crystallization

M_n (g/mol)	M_w/M_n	W_L (wt %)[a]	X_L (mol %)[a]	$[\alpha]^{25}_{598}$ (deg dm^{-1} g^{-1} cm^3)[a,b]	l_L[a]	l_C[a]	X_c (°C)	T_g (°C)	T_{cc} (°C)	T_m (°C)
5.91×10^4	2.1	77.2	84.3	−120.4	23.9	2.3	0	23.0	97.0	158.6

l_C = average CL unit sequence length value calculated from ^{13}C-NMR measurements; l_L = average L-lactyl unit sequence length value calculated from ^{13}C-NMR measurements; M_n = number-average molecular weight; M_w = weight-average molecular weight; T_{cc} = cold crystallization temperature; T_g = glass-transition temperature; T_m = melting temperature; W_L = weight of L-lactyl units; X_c = crystallinity; X_L = molar fraction of L-lactyl units.
  [a] The data were taken from ref. 36.
  [b] The specific optical rotation ($[\alpha]^{25}_{598}$) values were measured in chloroform.

amorphous, as evident from the broad diffraction profiles specific to amorphous polymeric materials. Surprisingly, the crystalline peak was observed for P(LLA-CL)-H film at a 2θ value of 16.5°, even at a short period of 6 h, and at 12 and 24 h, other crystalline peaks appeared at 2θ values of 19.0 and 22.4°, respectively. In contrast, the P(LLA-CL)-S films remained amorphous for 12 h, and at 24 h, a similar crystalline peak was observed for the P(LLA-CL)-S films at a 2θ value of 16.5°. The intensity of the crystalline peaks of the P(LLA-CL)-H films increased rapidly compared to that of the P(LLA-CL)-S films. These crystalline peaks observed for the P(LLA-CL)-H and P(LLA-CL) films were comparable to the ones for the α form of PLLA crystallized in a pseudo-orthorhombic unit cell of the dimensions: a = 1.07 nm, b = 0.595 nm, and c = 2.78 nm, which contained two 10₃ helices.[37] This confirmed that the

L-lactyl unit sequences of the copolymer formed crystallites. The crystallization of both the P(LLA-CL)-H and P(LLA-CL)-S films was due to the average length of L-lactyl unit sequences (23.9) in the copolymer, which was larger than the minimum length (15) for the crystallization of L-lactyl unit sequences in L-LA-rich copolymers synthesized from D-LA and D-LA.[38]

The crystallinity of the P(LLA-CL)-H and P(LLA-CL)-S films was estimated from the X-ray diffraction profiles,[39] and the obtained crystallinity values are plotted in Figure 3 as a function of crystallization time. The crystallinity values were higher for the P(LLA-CL)-H film than for the P(LLA-CL)-S film at the same crystallization times. The crystallinity of the P(LLA-CL)-H and P(LLA-CL)-S films increased rapidly from 1.5 to 19.5% and 4.5 to 18.1%, respectively, in the periods of 6–12 and 72–84 h. Then, the
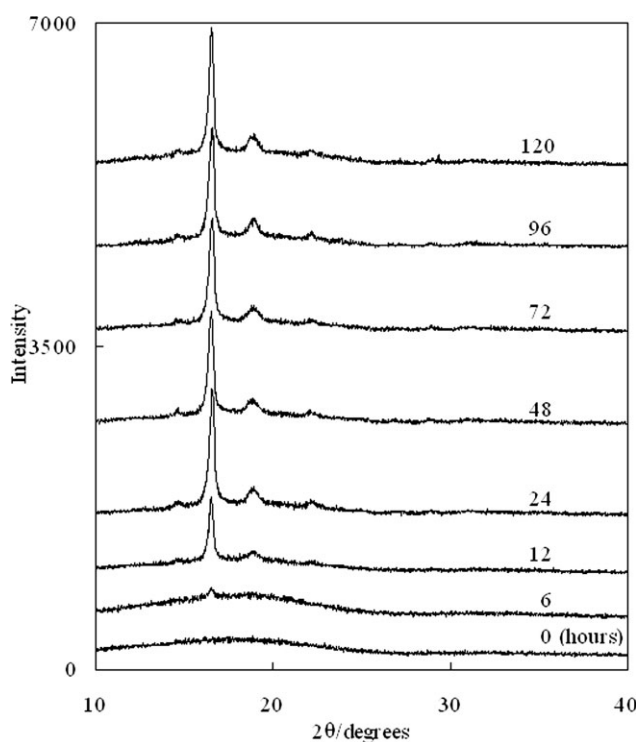

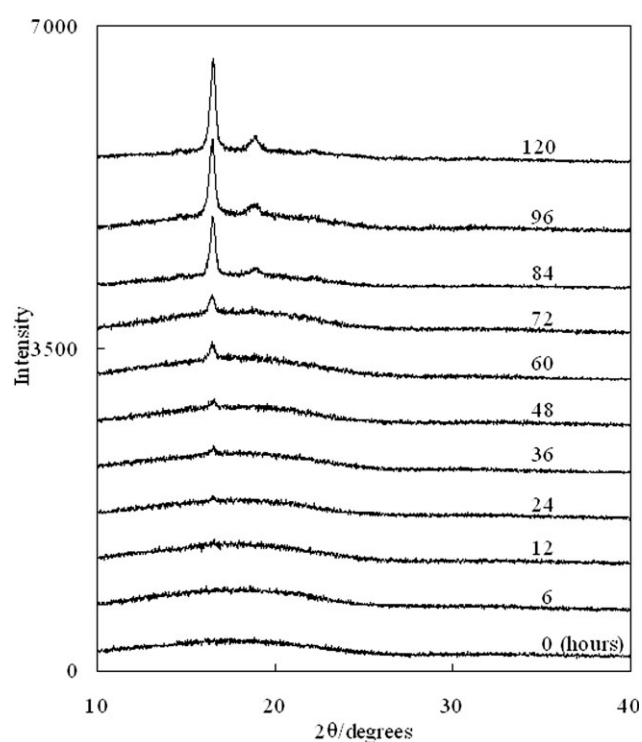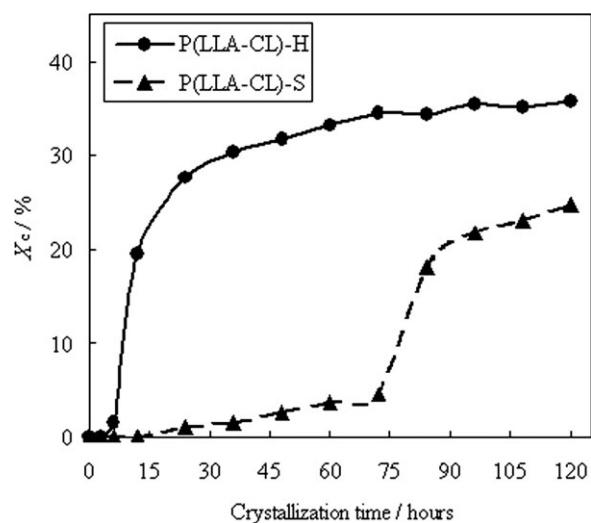Figure 1 X-ray diffraction profiles of P(LLA-CL)-H film after crystallization for different times.

Figure 2 X-ray diffraction profiles of P(LLA-CL)-S film after crystallization for different times.

Figure 3 Crystallinity (X_c) of P(LLA-CL)-H and P(LLA-CL)-S films estimated by X-ray diffraction as a function of the crystallization time.

crystallinity of the P(LLA-CL)-H and P(LLA-CL)-S films increased slowly with crystallization time and finally reached 35.8 and 24.8%, respectively, at 120 h. These findings reveal that the presence of water accelerated the crystallization of the P(LLA-CL)-H film compared to that of P(LLA-CL)-S film, probably because of the enhanced chain mobility induced by the plasticizing effect of water molecules. Plasticization, at a molecular level, leads to increased intermolecular space or free volume and may involve the weakening or breaking of selective interpolymer bonds, for example, polymer–polymer hydrogen bonding, and, hence, enhances chain mobility.[40,41] The change in the crystallinity of the P(LLA-CL)-H film was consistent with the reported result for an amorphous P(LLA-CL) (60/40) specimen, wherein a marked increase in crystallinity occurred within 48 h during hydrolytic degradation at 37°C.[26] The crystallization proceeded slowly in other L-LA copolymers in the presence of water. For example, the crystallinity of P(LLA-GA) (75/25) specimens increased from 6 to 23% in the periods of 7–9 weeks of hydrolytic degradation, as reported by Li et al.,[31] whereas Tsuji et al.[30] and Li et al.[29] observed that the crystallinity of amorphous PLLA films increased slowly from 4.7 to 21.8% and 4 to 17%, respectively, in periods of 52–104 and 18–31 weeks of hydrolytic degradation.

Morphology

The polarized optical photomicrographs of the P(LLA-CL) films after crystallization for 6, 24, and 120 h are shown in Figure 4. No spherulite (or assemblies of crystallites) was observed for the films before crystallization (photo not shown here), which revealed that the initial film was completely amor-

phous. At 6 h, some crystallites were observed for the P(LLA-CL)-H film [Fig. 4(A)]. In the period of 6–24 h, the crystallite number per unit area (density) increased in the P(LLA-CL)-H film [Fig. 4(C)]. In the period of 24–120 h, the crystallite density of the P(LLA-CL)-H films [Fig. 4(E)] remained unchanged, although the size of the crystallite assemblies increased. On the other hand, the P(LLA-CL)-S film [Fig. 4(B)] remained amorphous for 6 h. At 24 h, crystallites were observed for the P(LLA-CL)-S film [Fig. 4(D)]. In the period of 24–120 h, both the density and size of the crystallite assemblies increased [Fig. 4(F)]. All these photos support the X-ray results that the crystallization of P(LLA-CL) occurred in both the presence and absence of water. Moreover, as shown in Figure 4, the crystallite size and density of the P(LLA-CL)-H film were higher than those of the P(LLA-CL)-S film, when they were compared at the same crystallization periods. These findings indicate that the presence of water accelerated the crystallization of the P(LLA-CL)-H film more than that of the P(LLA-CL)-S film.

Mechanical properties

Table II shows the mechanical properties of the P(LLA-CL) films before and after hydrolytic degradation and storage, together with their molecular weight and thermal properties.[32] Here, we selected a crystallization period of 336 h (2 weeks) because below this period the period for complete drying of the P(LLA-CL)-H specimens (2 weeks) was much longer than the crystallization time, and the effect of further crystallization during drying should have been larger than that during crystallization. However, too long of a crystallization of P(LLA-CL)-H above 2 weeks in the presence of water caused significant hydrolytic degradation, as could be traced by molecular weight changes and, therefore, reduced the mechanical properties. The tensile strength and Young's modulus of the P(LLA-CL)-H and P(LLA-CL)-S films increased because of crystallization during hydrolytic degradation and storage. The elongation at break of the P(LLA-CL)-H and P(LLA-CL)-S films decreased rapidly to 19 and 23%, respectively, at 336 h, which were 36 and 44%, respectively, of the initial values, because of the formation of crystallites. These results indicate that the crystallization during hydrolytic degradation and storage enhanced the tensile properties of the P(LLA-CL) films, except for elongation at break. The changes in the tensile properties of the P(LLA-CL)-H and P(LLA-CL)-S films in this study were similar to those of the compression-molded P(LLA-CL) (60/40) specimen during hydrolytic degradation in phosphate buffered solution at 37 and 23°C reported by Karjalainen et al.[28] On the other hand, Tsuji et al.[26] observed
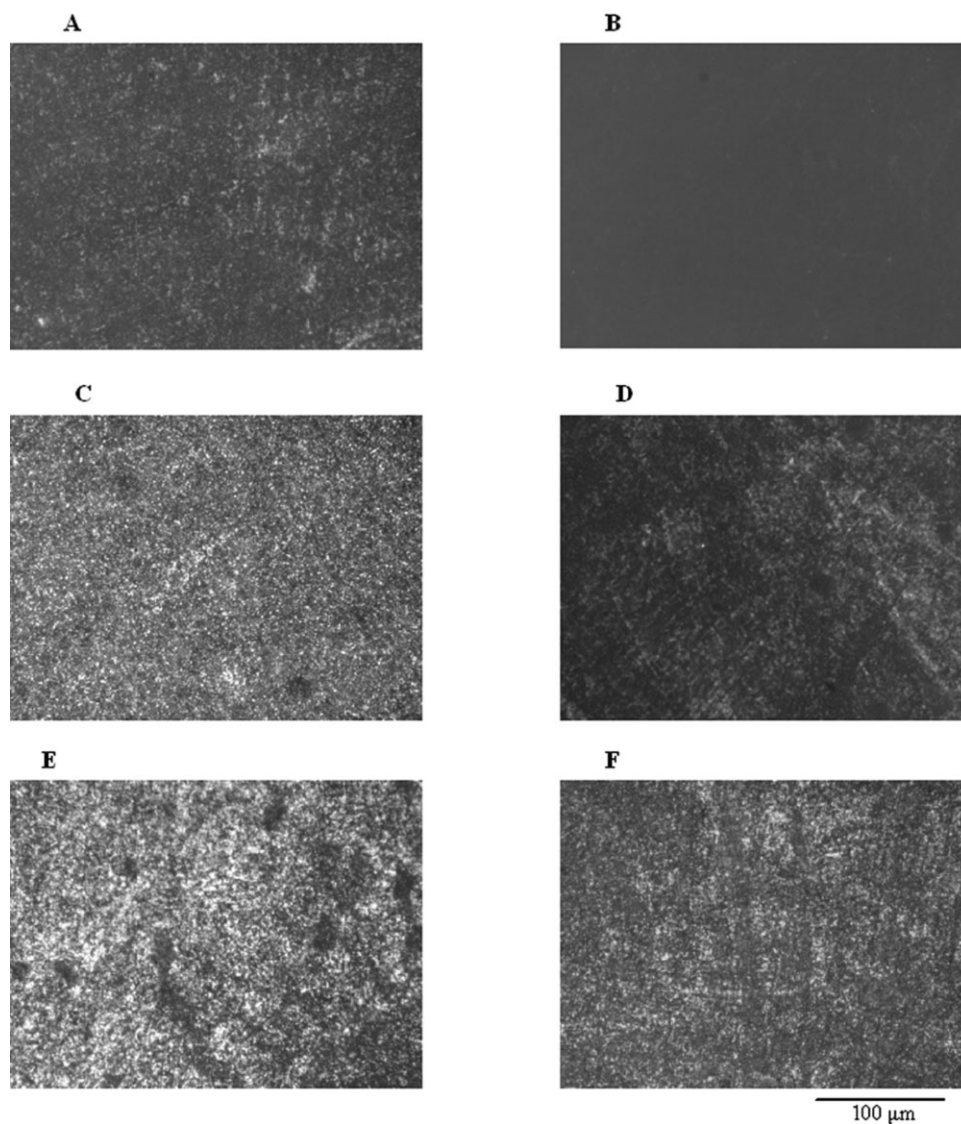
Figure 4 Polarized optical photomicrographs of (A,C,E) P(LLA-CL)-H and (B,D,F) P(LLA-CL)-S films crystallized in the presence or absence of water at (A,B) 6, (C,D) 24, and (E,F) 120 h.

similar changes in the tensile properties of a P(LLA-CL)(50/50) film during storage at room temperature for 60 days.

The properties required for polymers depend on their biomedical applications and purposes, where the mechanical performance and the degradation behavior are of great importance. One of the most crucial factors affecting the mechanical properties and degradation behavior of a degradable polymer is crystallinity. Crystallinity will affect the biological response, for example, cell growth and proliferation in the human body. It was reported that the

TABLE II
Mechanical Properties of the P(LLA-CL) Films Before and After Hydrolytic Degradation and Storage Together with Their Molecular Weights and Thermal Properties

Code	Crystallization time (h)	TS (MPa)	YM (MPa)	EB (%)	M_n (g/mol)	X_c (%)	T_m (°C)
P(LLA-CL)	0	6.3	239	53	6.37×10^4	0	159.3
P(LLA-CL)-H	336	9.6	263	19	5.28×10^4	28.5	160.0
P(LLA-CL)-S	336	11.8	287	23	6.25×10^4	26.1	159.4

The data were taken from ref. 32. EB = elongation at break; M_n = number-average molecular weight; T_m = melting temperature; TS = tensile strength; X_c = crystallinity; YM = Young's modulus.

hydrolyzability of PLLA in neutral media increases with the cyrstallinity.[24,25] The results in this study strongly suggest that the presence of water can accelerate the crystallization (and, therefore, the mechanical property changes) of crystallizable biodegradable materials, that treatment in the presence of water can be used for rapid structural stabilization of biodegradable materials, and that the crystallizability of biodegradable materials in a relatively long period during the storage in the absence of water can be predicted from the results in a short period in the presence of water. Therefore, the information obtained in this study would be useful for the selection, preparation, and use of biodegradable materials in biomedical applications.

CONCLUSIONS

The following conclusions can be derived from the aforementioned results for the crystallization of amorphous-made P(LLA-CL) films in the presence and absence of water. The crystallinity of P(LLA-CL) dramatically increased in 6–12 and 72–84 h in the presence and absence of water, respectively, and, after that, slowed down. This revealed that the presence of water accelerated the crystallization of the copolymer, probably because of enhanced chain mobility. Such a rapid increase in crystallinity is anticipated to cause the dramatic changes in the mechanical properties and hydrolytic degradation behavior of P(LLA-CL).

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