Decomposition Rate Control of PLLA Plate by Heat Treatment

Masafumi Nakagawa, Fumio Teraoka, Taiji Sohmura

Division of Biomaterial Science, Graduate School of Dentistry, Osaka University, Osaka 565-0871, Japan

Received 22 June 2009; accepted 4 June 2010
DOI 10.1002/app.32931
Published online 25 August 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: It is difficult to control the decomposition rate and the mechanical property of scaffolds after forming the poly(L-lactide) (PLLA) scaffolds. The purpose of this study is to control the decomposition rate and mechanical properties of the PLLA plate after forming. We carried out accelerated decomposition experiments using the enzyme on the (PLLA) with various crystallinity, which were prepared by changing the heat treatment condition, and elucidated the relationship between the crystallinity and the decomposition rate. A high positive correlation was observed between the heat treatment temperature and the crystallinity. A high negative correlation was observed between the crystallinity and the decomposition rate. Using the obtained empirical formula, it became possible to calculate the required period to decompose a certain amount of the PLLA if the heat treatment temperature was known. Changing the crystallinity of the PLLA plate could arbitrarily control the decomposition rate of the PLLA plate after forming. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2031–2038, 2011

Key words: decomposition rate; poly(L-lactide) (PLLA) plate; heat treatment; crystallinity; flexural strength

INTRODUCTION

When a body tissue is slightly damaged, the tissue is naturally self-regenerated. However, when the tissue is severely damaged or removed that exceeds a critical value, it is difficult to completely self-regenerate the tissue. In such a case, one must stimulate the tissue to regenerate by putting an artificial scaffold on the defective part.[1–3] That is, the scaffold plays a significant role in the tissue engineering.[4–10] Ideally, the scaffold should be gradually absorbed as the tissue is regenerated in the scaffold and should be completely absorbed and replaced with the tissue when the tissue is completely regenerated.

Concerning the biodegradable polymer, though there are many reports on the control of the decomposition rate by blending[11–14] or by using a copolymer,[15–18] there is only a few reports on the control of the decomposition rate after forming the scaffold. It is expected that the scaffold will have a much broader range of application in clinical treatments if the decomposition rate of the scaffold can be controlled after forming. As for the studies concerning the decomposition of the biodegradable polymer, though there were many reports on hydrolysis,[15,17,19,20] examinations of the decomposition using the enzyme have been recently reported.[21–24] The purpose of our study is to control the decomposition rate of the biodegradation polymer plate after forming. In this study, we carried out an accelerated decomposition examination using the enzyme for the injection-molded poly(L-lactide) (PLLA) plates, which had various crystallinities by applying heat treatment, and elucidated the relationship between the crystallinity, the decomposition rate, and the mechanical properties.

Correspondence to: F. Teraoka (terachan@dent.osaka-u.ac.jp).

Journal of Applied Polymer Science, Vol. 119, 2031–2038 (2011)

MATERIALS AND METHODS

Preparation of specimen

Poly(L-lactide)(PLLA) with an average molecular weight (M_w) of 220,000 g/mol and the polydispersity (M_w/M_n) of 2.3 was obtained from a commercial source (Lacty 5000, Shimazu, Kyoto, Japan) in pellet form. Specimens having a size of 60 mm × 10 mm × 2.5 mm were prepared by melting the pellets in a metal cylinder at 215°C for 30 min and then injecting (Sulfon-Jet 3000, High-Dental-Japan, Osaka, Japan) it into a metal mold cooled to 4°C. The injection molding was carried out at an injection pressure of 15 MPa. The molded specimens were preserved in a desiccator at 23°C until used. These specimens were labeled PLLA-4.

The PLLA-4 injection-molded specimens were heat-treated for one hour at 75, 80, 100, and 140°C. These specimens were labeled PLLA-75, PLLA-80, PLLA-100, and PLLA-140, respectively. Table I shows the preparation condition and abbreviations of five different samples.

TABLE I
Preparation Condition and Abbreviations of Five Different Samples

Melting condition		Injection condition		Heat treatment condition		
Temperature (°C)	Time (min)	Mold temperature (°C)	Pressure (MPa)	Temperature (°C)	Time (h)	Abbreviation
215	30	4	15	Non	Non	PLLA-4
215	30	4	15	75	1	PLLA-75
215	30	4	15	80	1	PLLA-80
215	30	4	15	100	1	PLLA-100
215	30	4	15	140	1	PLLA-140

Decomposition experiment of PLLA

The enzyme solution was prepared by mixing 10 mL of Tris-HCl (Sigma, MO), 2 mg of proteinase K (Sigma, MO), and 2 mg of sodium azide (Nacalai Tesque, Kyoto, Japan), and by adjusting the pH to 8.6. The decomposition experiment was carried out by placing the specimen in the enzyme solution and keeping the mixture for 1, 2, 3, 7, and 14 days while rotating with a bio-shaker (BR-15LF, Taitec, Saitama, Japan) at the rate of 130 rpm at 37°C. The weight loss after the decomposition was obtained using the following equation. The weight after the immersion was measured after it had dried for 24 h.

$$\text{Weight loss } (\%) = (W_0/W_t) \times 100$$

where W_0 and W_t are the weight before and after immersion, respectively. Five specimens were prepared for each specimen before and after the heat treatment and for each immersion period with a total number of 125 specimens.

X-ray diffraction analysis

The X-ray diffraction patterns of the specimen before and after the heat treatment were obtained using an X-ray diffractometer (Rint 2000, Rigaku, Tokyo, Japan) at an acceleration voltage of 20 kV, tube current of 10 mA.

Difference scanning calorimetry (DSC)

The thermal analysis of each specimen was carried out using thermal analysis equipment (DSC-60, Shimadzu, Kyoto, Japan) at the rate of temperature rise of 10°C/min. The crystallinity of each specimen was obtained using the following equation.[25]

$$X_c = 100 \cdot (\Delta H_m + \Delta H_c)/93$$

where X_c, ΔH_c, and ΔH_m indicate the crystallinity, crystallization enthalpy, and fusion enthalpy, respectively. Five specimens were prepared for each specimen before and after the heat treatment and for each immersion period with a total number of 150 specimens.

Flexural test

The flexural test of each sample before and after the heat-treatment and immersion in the enzyme solution was carried out using a testing machine (EZ-Test, Shimadzu, Kyoto, Japan). The shape of the specimen was 60 mm × 10 mm × 2.5 mm, and the three-point flexural test was done for a distance between the supporting points of 50 mm and the crosshead speed of 5 mm/min. Five specimens were prepared for each specimen before and after the heat treatment and for each immersion period with a total number of 150 specimens. Additionally, five compression-specimens were prepared for comparison. The compression-specimens having a size of 60 mm × 10 mm × 2.5 mm were prepared by melting the pellets in a metal mold at 215°C for 35 min and then pressing (Bench press 100, Imoto, Kyoto, Japan) it at a compression load of 1 kN.

Surface observations

The surface of the specimen before and after immersion in the enzyme solution was observed in the dynamic mode using an atomic force microscope (SPM-9500, Shimadzu, Kyoto, Japan).

Statistical analysis

The data obtained from each test were statistically treated. The mean value and the standard deviation were calculated. Fisher's multiple comparison tests were carried out after the significance test by the analysis of variance (ANOVA). They were statistically processed at the significance level of 5%. The percentage of risk ($P < 0.05$) for the correlation coefficient and the P-value was obtained by Spearman's rank correlation.

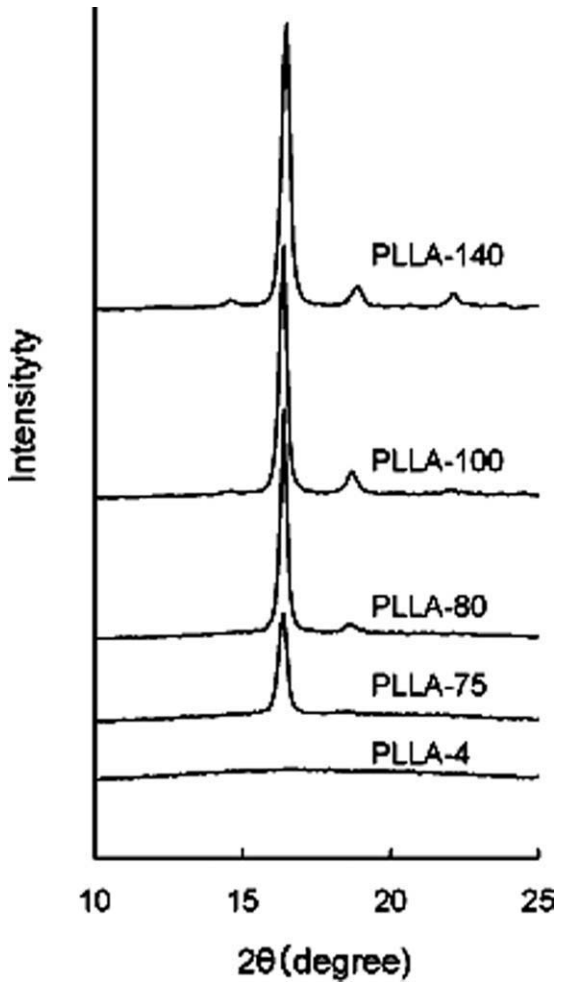

Figure 1 X-ray diffraction patterns before and after the heat treatment. No peak was observed in the X-ray diffraction pattern of PLLA-4. However, a peak around $2\theta = 16.5°$ became sharper as the heat-treatment temperature increased.

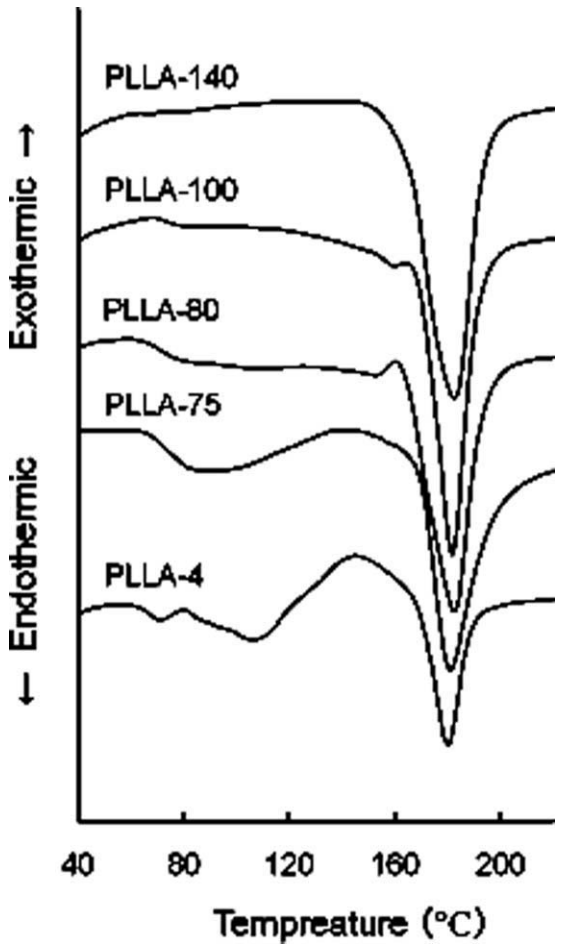

Figure 2 DSC curves before and after the heat treatment. An exothermic peak and a melting peak due to the glass transition point (T_g) and recrystallization were observed for PLLA-4, PLLA-75, PLLA-80, and PLLA-100, only the melting peak was observed for PLLA-140.

RESULTS

Crystallization and crystallinity

Though no peak was observed in the X-ray diffraction pattern for PLLA-4, a peak near $2\theta = 16.5°$ appeared for the heat-treated samples, and the peak became sharper as the heat treatment temperature increased (Fig. 1). The DSC curves of PLLA before and after the heat treatment are shown in Figure 2. PLLA-4 had a glass transition point (T_g) at about 55°C, and an exothermic peak due to recrystallization and a melting peak were observed. The T_g became unclear as the heat treatment temperature increased, and the recrystallization peak also decreased. As for PLLA-140, the T_g and the recrystallization peak disappeared, and only the melting peak was observed. The crystallinity of PLLA-4, PLLA-75, PLLA-80, PLLA-100, and PLLA-140 were 0 ± 0, 14.3 ± 2.0, 21.7 ± 2.2, 41.7 ± 3.6, and $62.9 \pm 3.2\%$, respectively, and a high positive correlation was observed between the heat treatment temperature and the crystallinity (Fig. 3). No significant change in the crystallinity was observed for PLLA-4,

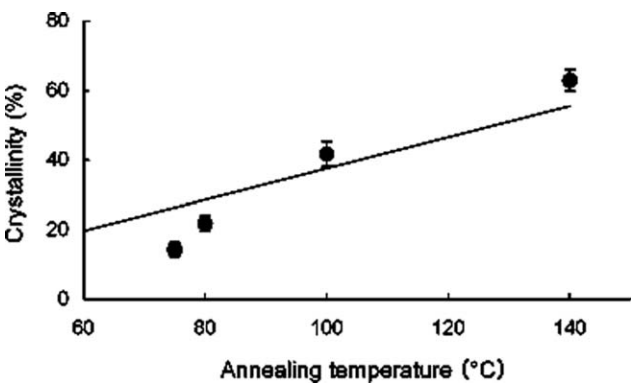

Figure 3 Relationship between heat-treatment temperature and crystallinity ($n-5$). A high positive correlation was observed between the heat treatment temperature and the crystallinity. $X_c = 0.60T–22.79$ ($r = 0.97$).

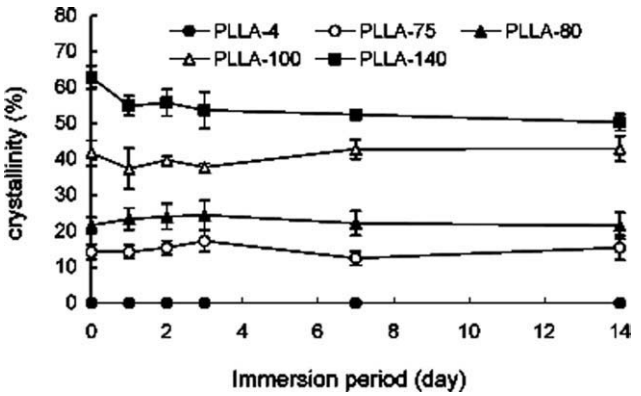

Figure 4 Changes in the crystallinity for the specimen immersed in enzyme solution ($n-5$). No significant change in the crystallinity was observed in the specimens other than PLLA-140 even though they had been immersed in the enzyme solution.

PLLA-75, PLLA-80, and PLLA-100 even if they had been immersed in the enzyme solution (Fig. 4). However, as for PLLA-140 immersed for one day, the crystallinity significantly decreased compared to the specimen before immersion and gradually decreased thereafter (Fig. 4). Moreover, the X-ray diffraction peak of PLLA-140 became smaller as the immersion period in the enzyme solution became longer (Fig. 5) and also the T_g appeared (Fig. 6).

Weight change

The weight of each specimen decreased almost linearly as the immersion period in the enzyme solution became longer (Fig. 7). The weight losses of PLLA-4, PLLA-75, PLLA-80, PLLA-100, and PLLA-140 after immersion for 14 days were 18.86 ± 0.58, 15.89 ± 2.09, 13.54 ± 0.87, 4.05 ± 1.46, and 2.40 ± 1.58 wt %, respectively, and a significant difference was observed. Moreover, the decomposition rates of PLLA-4, PLLA-75, PLLA-80, PLLA-100, and PLLA-140 obtained from the inclination of the line were 1.35, 1.11, 0.93, 0.30, and 0.18 wt %/day, respectively, and a high negative correlation was observed between the crystallinity and the decomposition rate (Fig. 8).

Flexural strength

The flexural strengths of PLLA-4, PLLA-75, PLLA-80, PLLA-100, and PLLA-140 were 112.3 ± 8.2, 123.8 ± 8.4, 134.0 ± 5.7, 141.2 ± 11.4, and 95.8 ± 8.4 MPa, respectively (Fig. 9). No significant difference was observed in the flexural strength of PLLA-4 before and after immersion. The flexural strength of PLLA-75, immersed for two days or more, significantly decreased compared to the specimen before immersion. On the other hand, the flexural strength of the PLLA-80, PLLA-100, and PLLA-140, immersed for one day or more, significantly decreased compared to the specimens before immersion.

Surface condition

Figure 4 shows the AFM images of the specimens before and after immersion in the enzyme solution for 14 days. A grinding mark from the metal mold was observed on the surface of the injection-molded specimen, and almost no change was observed after the heat treatment [Fig. 10(a,c,e)]. As for the surface of PLLA-4 immersed in the enzyme solution, crater-shaped pits were observed, and their depths were 200–300 nm [Fig. 10(b)]. On the other hand, a partly eroded Grand Canyon-shaped structure was

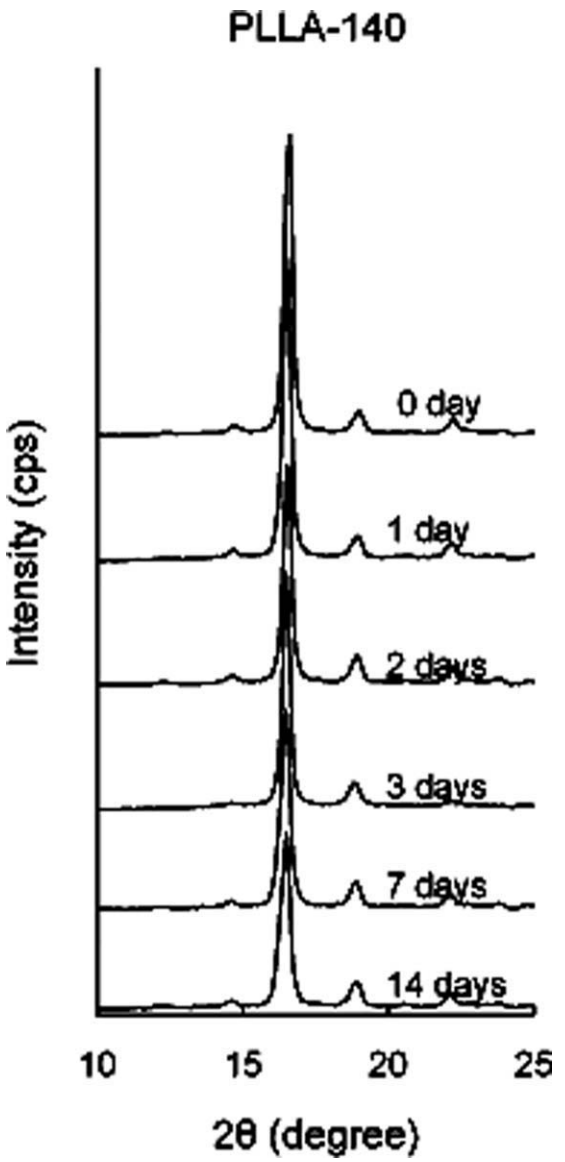

Figure 5 X-ray diffraction patterns of PLLA-140 immersed in enzyme solution. The X-ray diffraction peak of PLLA-140 decreased as the immersion period in the enzyme solution increased.

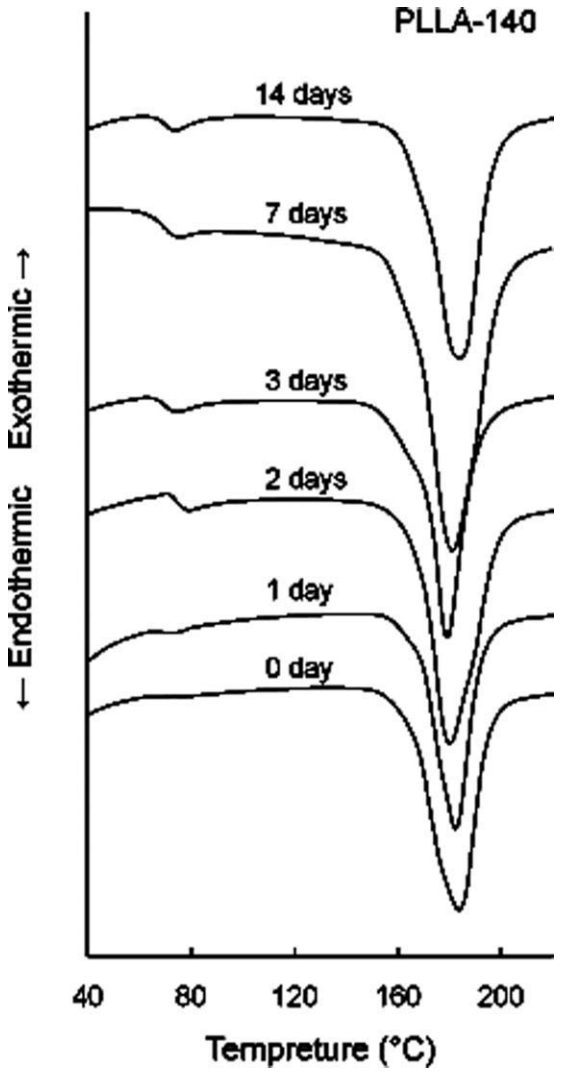

Figure 6 DSC curves of PLLA-140 immersed in enzyme solution. The T_g appeared in the DSC curve of PLLA-140, which was immersed in the enzyme solution.

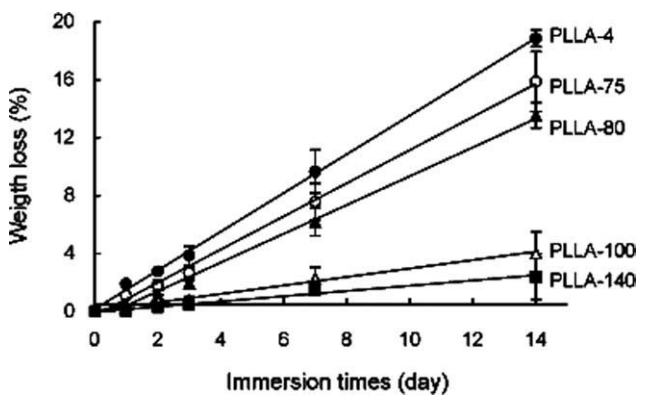

Figure 7 Weight losses of the specimen immersed in enzyme solution (n−5). The weight of each specimen immersed in the enzyme solution almost linearly decreased along with the increase in the immersion period.

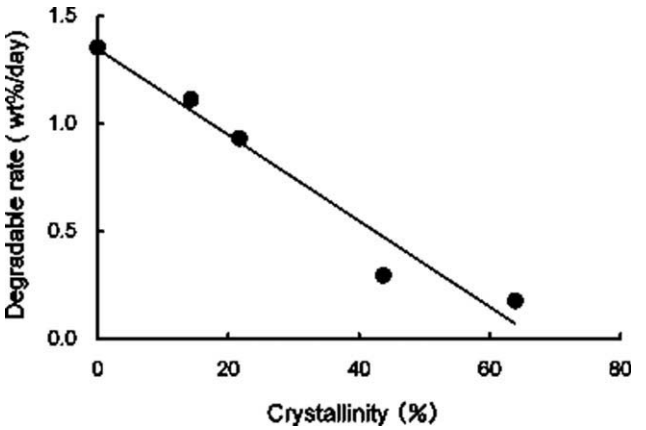

Figure 8 Relationship between crystallinity and decomposition rate. A high negative correlation was observed between the crystallinity and the decomposition rate. W/d $= -0.02\ X_c + 1.35$ ($r = 0.98$).

observed for PLLA-80 and PLLA-140, and the largest depth of the eroded part was about 1.5 and 2.5 μm, respectively [Fig. 10(d,f)].

DISCUSSION

The heat treatment time is a factor that also influences the crystallization as well as the heat treatment temperature.[26] We carried out an experiment using the constant heat treatment time of 1 h based on a preliminary experiment. Though PLLA-4 was almost in an amorphous state, the crystallinity linearly increased along with the increase in the heat treatment temperature. Because the T_g and the recrystallization peak were hardly detected in the DCS-curve of PLLA-140, it can be regarded that the crystallization was almost completed under the heat treatment condition of 140°C for 1 h. A high correlation was

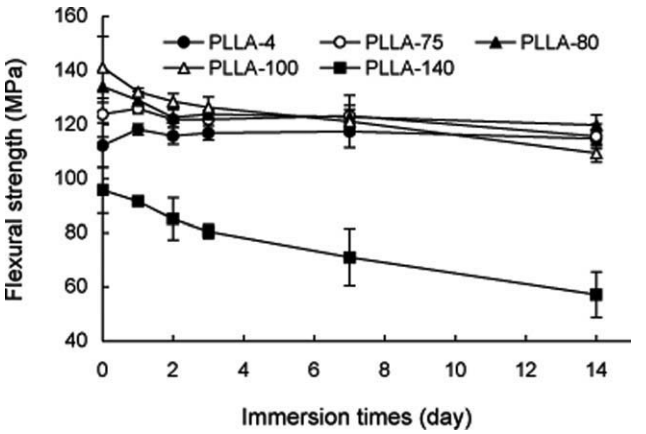

Figure 9 Flexural strength of the specimen immersed in enzyme solution (n−5). Though no significant difference was observed in flexural strength for the PLLA-4 specimens before and after immersion, the flexural strength of the heat-treated specimen, which was immersed for one or two days or more, significantly decreased compared to the specimen before immersion.

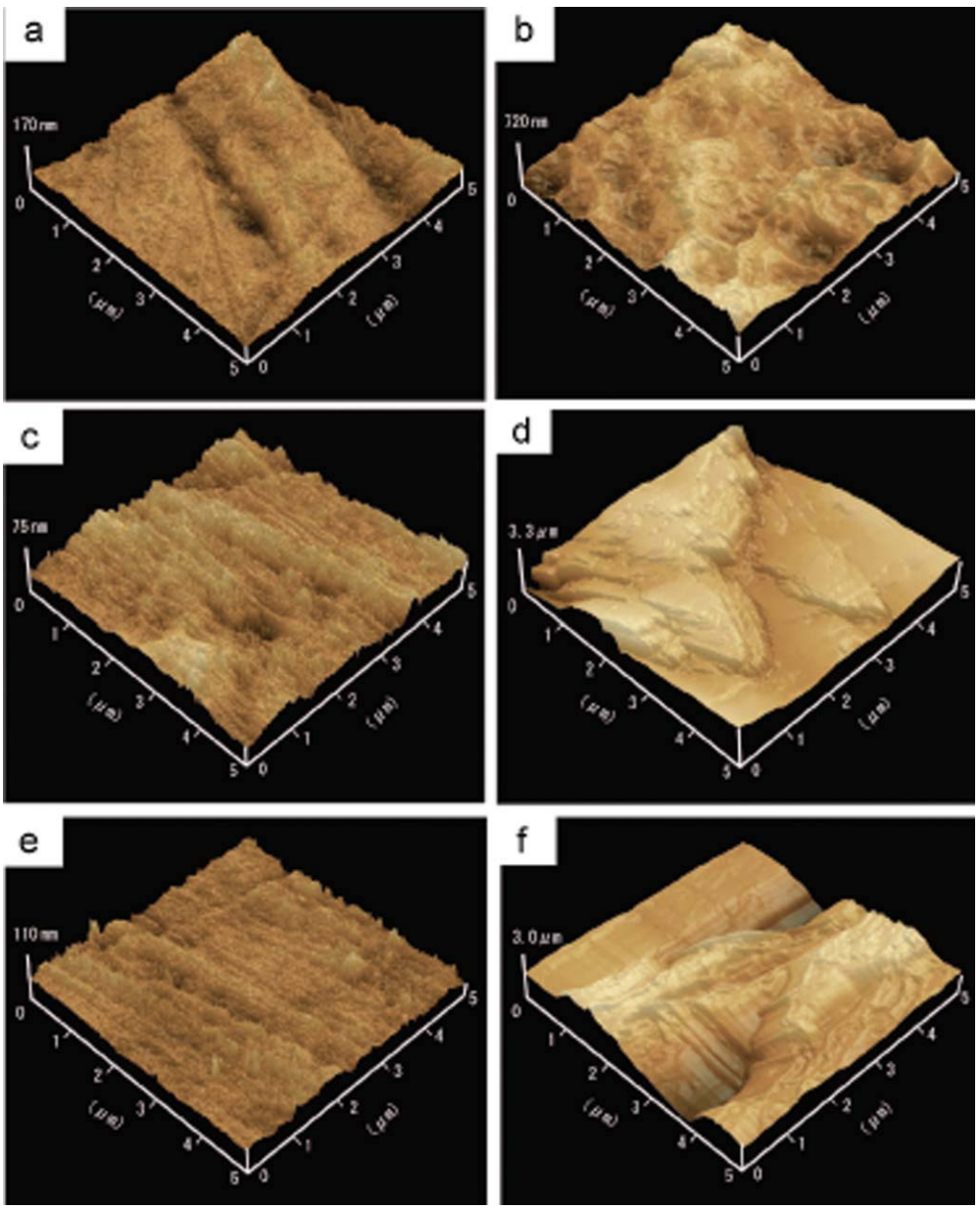

Figure 10 AFM images of specimen before immersion in the enzyme solution (a, c, and e) and AFM images of specimen after immersion in the enzyme solution for 14 days (b, d, and f). Changes in the surface of the specimen before and after the heat treatment were hardly observed (a, c, and e). Crater-shaped pits were observed on the surface of PLLA-4 (b), which had been immersed in the enzyme solution, and a partly eroded Grand Canyon-shaped surface was observed for PLLA-80 (d) and PLLA-140 (f). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed between the heat treatment temperature and the crystallinity, and the following empirical formula was obtained.

$$X_c = 0.45T - 7.34 \quad (r = 0.95) \tag{1}$$

where X_c, T, and r are the crystallinity, the heat treatment temperature, and the correlation coefficient, respectively.

The amount of decomposition almost linearly increased along with the increase in the immersion period. The higher the crystallinity of the specimen, the lower the decomposition rate. A high negative correlation was observed between the crystallinity and the decomposition rate, and the following empirical formula was obtained.

$$W/d = -0.02X_c + 1.35 \quad (r = 0.98) \tag{2}$$

where W, d, and W/d are the amount of decomposition (wt %), the decomposition period (day), and the decomposition rate (wt%/day), respectively. Equations (3) and (4) are obtained by substituting eq. (1) into equation eq. (2).

$$T = -111.11(W/d) + 166.31 \quad (3)$$

$$d = -111.11W/(T - 166.31) \quad (4)$$

When the desired amount of decomposition and decomposition period are substituted into eq. (3), the required heat treatment temperature is obtained. For instance, the heat treatment temperatures for preparing the specimens, which decompose by 20% after 30 days, 40% after 100 days, and 50% after 200 days, were determined to be 92.2, 121.9, and 138.5°C, respectively. Conversely, if the heat treatment temperature is known, the decomposition period required for achieving the specified amount of decomposition is obtained using eq. (4). For instance, the periods when the amount of decomposition becomes 50% for each PLLA-4, PLLA-75, PLLA-80, PLLA-100, and PLLA-140 are 33.4, 60.8, 64.4, 83.8, and 211.2 days, respectively. Because the crystallization by the heat treatment hardly proceeds below the T_g, the minimum heat treatment temperature is about 55°C. Moreover, because the deformation of the specimen takes place near its melting point, the maximum heat treatment temperature is about 140°C. Based on the above results, the condition for eq. (3) to be true is $55 < T < 140$.

The flexural strength of the specimen increased along with an increase in the heat treatment temperature in the range below 100°C. It was found that the crystallinity contributed to the improvement of the flexural strength. However, the flexural strength of PLLA-140 significantly decreased compared with the other specimens. In the case of PLLA-140, the amount of shrinkage in the long axis direction was greater than that of the other heat-treated specimens. The flexural strength of the specimen, which had been compression-molded in a metal mold, was 94.5 MPa. It was significantly low compared to the value of 141.1 MPa for PLLA-4, which had been injection-molded. From this, it is understood that the flexural strength was also influenced by the molecular orientation as well as the crystallinity. However, no structural and appearance changes were observed in all of the samples except for the PLLA-140.

We carried out the accelerated decomposition experiment using the enzyme in this experiment. PLLA-4 consisted mostly of an amorphous region, and its decomposed surface had crater-shaped pits with depths of 200–300 nm. It means that an almost uniform decomposition occurred. However, as for the partially crystallized specimen, a nonuniform decomposition occurred, and a Grand Canyon-shaped surface was observed. These decomposed parts are amorphous and these results coincided with the report that proteinase K used in this experiment decomposed the amorphous region of PLLA.[27,28] However, because the T_g was observed in the DSC for the decomposed PLLA-140, which had the maximum crystallinity, it is understood that the amorphous region increased due to the immersion in the enzyme solution. From this, it is presumed that though the decomposition by the enzyme occurred in the amorphous region, the crystalline region was not directly decomposed, but decomposed after it became an amorphous region. Duek et al.[29] reported that the crystallization along with the decomposition proceeded during the hydrolysis of the amorphous PLLA. However, no increase in the crystallinity was observed in the decomposition process during this experiment using the enzyme. It is presumed that it depends on the difference in the decomposition mechanism between the hydrolysis and the decomposition with the enzyme.

We found that the arbitrary amount of decomposition and decomposition period could be obtained by a prior calculation. Furthermore, the amount of decomposition in separate areas can be individually controlled if the heat treatment temperature is changed corresponding to the area of the PLLA plate, therefore, the application range of this study will be extended. Moreover, a scaffold, which has a gradient in its decomposition rate, can be prepared if the heat treatment is applied with a temperature gradient from the upper part to the lower part of the scaffold. Therefore, our method is expected to have a wide range of applications.

CONCLUSIONS

The purpose of this study is to control the decomposition rate and mechanical properties of the PLLA plate after forming. The crystallinity of the specimen increased with the heat treatment temperature, and a high positive correlation was observed between the heat treatment temperature and the crystallinity. No significant change in the crystallinity was observed for the specimens except for the PLLA-140 even if they had been immersed in the enzyme solution. The weight of each specimen decreased almost linearly as the immersion period in the enzyme solution became longer. A high negative correlation was observed between the crystallinity and the decomposition rate. The flexural strength of the specimens expect for the PLLA-4, immersed for two days or more, significantly decreased compared to the specimens before immersion. Using the obtained empirical formula, it became possible to calculate the required period to decompose a certain amount of the PLLA if the heat treatment temperature was known. Changing the crystallinity of the PLLA plate could arbitrarily control the decomposition rate of the PLLA plate after forming.

References

1. McNamara, A.; Williams, D. F. Biomaterials 1981, 2, 33.
2. Vince, D. G.; Hunt, J. A.; Williams, D. F. Biomaterials 1991, 12, 731.
3. Honkanen, P. B.; Kellormåki, M.; Lehtimåki, M. Y.; Tørmålå, P.; Måkelå, M. U. Tissue Eng 2003, 9, 957.
4. Langer, R.; Vacanti, J. P. Science 1993, 260, 920.
5. Reddi, A. H. Tissue Eng 2000, 6, 351.
6. Stock, U. A.; Vac, J. P. Annu Rev Med 2001, 52, 443.
7. Rose, F. R. A. J.; Oreffo, R. O. C. Biochem Biophys Res Commun 2002, 292, 1.
8. Young, C. S.; Terada, S.; Vacanti, J. P.; Honda, M.; Bartlett, J. D.; Yelick, P. C. J Dent Res 2002, 81, 695.
9. Schmidt, C. E.; Leach, J. B. Annu Rev Biomed Eng 2003, 5, 293.
10. Allen, K. D.; Athanasiou, K. A. J Dent Res 2008, 87, 180.
11. Calandrelli, L.; Immirzi, B.; Malinconico, M.; Volpe, M. G.; Oliva, A.; Ragione, F. D. Polymer 2000, 41, 8027.
12. Liu, B.; Du, Q.; Yang, Y. J Membr Sci 2000, 180, 81.
13. Zeng, M.; Fang, Z.; Chengwei, C. J Membr Sci 2004, 230, 175.
14. Yu, L.; Dean, K.; Li, L. Prog Polym Sci 2006, 31, 576.
15. Du, J.; Zheng, Y.; Xu, L. Polym Degrad Stab 2006, 91, 3281.
16. Jung, Y. M.; Kim, H. J.; Ryu, D. Y.; Kim, S. B.; Kim, J. K. J Mol Struct 2006, 799, 96.
17. Luckachan, G. E.; Pillai, CKS. Carbohydr Polym 2006, 64, 254.
18. Mothé, C. G.; Drumond, W. S.; Wang, SH. ThermoChim Acta 2006, 445, 61.
19. Jang, W.; Lee, C.; Sundar, S.; Shul, Y. G.; Han, H. Polym Degrad Stab 2005, 90, 431.
20. Lee, C. F.; Yang, C. C.; Wang, L. Y.; Chin, W. Y. Polymer 2005, 46, 5514.
21. Sundback, C. A.; Shyu, J. Y.; Wang, Y.; Faquin, W. C.; Langer, R. S.; Vacanti, J. P.; Hadlock, T. A. Biomaterials 2005, 26, 5454.
22. Kurokawa, K.; Yamashita, K.; Doi, Y.; Abe, H. Polym Degrad Stab 2006, 91, 1300.
23. Bourissou, D.; Sanchez, S. M.; Vaca, B. M. C R Chim 2007, 10, 775.
24. Zhao, Z.; Yang, L.; Hu, Y.; He, Y.; Wei, J.; Li, S. Polym Degrad Stab 2007, 92, 1769.
25. Fisher, E. W.; Sterzel, H. J.; Wegner, G. Kolloid Z Polym 1973, 251, 980.
26. Lorenzo, M. L. D.; Silvestre, C. Prog Polym Sci 1999, 24, 917.
27. Tsuji, H.; Miyauchi, S. Polymer 2001, 42, 4463.
28. Okada, M. Prog Polym Sci 2002, 27, 87.
29. Duek, E. A. R.; Zavaglia, C. A. C.; Belangero, W. D. Polymer 1999, 40, 6465.