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# Degradation of morpholine-2, 5-dione derivative copolymer in Vitro

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## Summary

Degradation of Poly (L-lactide-co-glycolic acid-co-β-Benzyl-L-aspartate) [P(LLA-BMD)] in vitro was carried out in phosphate buffer solution (PBS) at 37°C for 90 days. Poly (L-lactide-co-glycolide) (PLGA) was used as the control sample. Using gravimetric (weight loss) method, scan electron microscope (SEM), FT-IR, $^1$H-NMR, Gel permeation chromatography (GPC) and contact angel test as the testing methods, the degradation properties of this copolymer were obtained. The results showed that the copolymer of morpholine-2,5-dione derivative and L-lactide had a lower contact angel (74°) and slower degradation rate than that of PLGA. Under the current experimental conditions the degradation mechanism of P(LLA-BMD) is probably bulk degradation, being the same as PLGA. And the degradation of P(LLA-BMD) was more easily to be under control than PLGA, what's more, the mole composition had hardly changed ( from $n_{LLA}$:$n_{BMD}$=83/17 to 85/15 in 90 days). It would be served as a potential candidate material in the delivery of water-soluble drugs.

## Introduction

Synthetic biodegradable, bioresorbable polyesters, such as poly (L-lactide) and poly (ε-caprolactone) *et al*[1-3], having good biocompatibility and biodegradation, have been widely used in tissue engineering and pharmaceutical applications. However, these polyesters have no functional side groups in the main chain and because of their poor hydrophilicity, the biodegradation of these polymers was difficult to be under control. In order to improve the hydrophilicity of these polyesters, one of the promising methods is to introduce hydrophilic units into the copolymer chains [4-6]. In this paper, (3s)-[(benzyloxycarbonyl) methyl] morpholine-2,5-dione(BMD) was synthesized and it was copolymerized with L-lactide (L-LA). Using PLGA as the control sample, we obtained degradation property of this copolymer.

## Experiment

### *Materials*

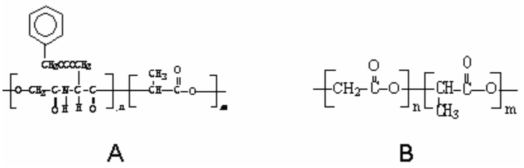

**Fig. 1** Chemical structure of copolymers (A) P(LLA-BMD) (B) PLGA

Poly(L-lactide-co-glycolide) (PLGA, $n_{LLA}/n_{GA}$=80/20, Mn=8.4×$10^4$) was obtained by ring opening polymerization of L-lactide (Purac) and glycolide (Purac) in bulk at 140°C, using stannous -2-ethylhexane (Sigma-Aldrich) as the catalyzer. Poly(L-lactide-co-glycolic acid-co-β-Benzyl-L-aspartate) [P(LLA-BMD), $n_{LLA}/n_{BMD}$=83/17] with different molecular weight (Mn=8.6×$10^4$, 6.5×$10^4$) was prepared as described in ref.[7]. Chloroform, sodium hydrate and sodium phosphates were purchased from Shanghai Chemical Reagent Co. and used without further purification.

### *Instruments*

The morphological features of the films were investigated by scanning electron microscope (SEM, JSM-6360, JP.). Gel permeation chromatography (GPC) was carried out on a Waters HPLC system equipped with a Model 2690D separation module, a Model 2410 refractive index detector, and Shodex columns (K802.5, K803, and K805). Chloroform was used as eluent at a flow rate of 1.0 ml/min. Calibration was fulfilled with narrow-molar-mass distributed polystyrene standards. The infrared spectral analysis of the samples was performed on a FT-IR spectrophotometer (Nicolet Magna-IR550). $^1$H-NMR spectroscopy was performed on a Bruker DRX-500 spectrometer. $CDCl_3$ was used as the solvent. Tetramethylsilane was used as an internal standard. The static water contact angle was measured by using a static drop method at 25°C±0.1°C with a Drop Shape Analysis System (JC2000A. Shanghai Powereach Digital Equipment .Ltd. Co.). Each sample was measured for three times.

### *Preparation of copolymer films*

Approximately 0.40 mm thick films of two copolymers were fabricated through casting 4% (w/v) solutions of chloroform onto polytetrafluoroethylene (PTFE) plates. Solvent evaporation was allowed to take place at room temperature for 24h. The residual solvent was removed by heating the films under vacuum at 50°C for 6h. Films with about 10*30 mm (width*length) were used in vitro degradation experiments.

### *Water absorption*

The films (initial weight: $m_0$) were immersed into PBS for 30 min at 37°C, then taken out and dried by the absorbent paper. Then the films were weighed again to obtain the weight with water ($m_1$). Then water absorption rate ($W_A$) was calculated according to equation (1).

$$W_A=(m_1-m_0)/m_0*100\% \qquad \textbf{(1)}$$

*Degradation in PBS*

The films of PLGA and P(LLA-BMD) were immersed into the glass vessels containing 5 mL of pH=7.4 phosphate buffer solution (0.2 M). The glass vessels were maintained in the culture set for a predetermined period of time at 37°C, exchanging the buffered solution each 2 days. After a determined period of time, the films were withdrawn, and washed with distilled water, wiped by the absorbent paper, then dried in vacuum to a constant weight at room temperature (25°C). Finally the surface morphological features, weight loss, molecular weight remaining, polydispersity index and mole composition of the copolymers were analyzed.

*Weight loss*

Weight loss rate ($W_L$) was calculated according to equation (2).

$$W_L= W_l/W_i *100\% \qquad \textbf{(2)}$$

$W_i$: initial weight, $W_l$: lost weight

*Molecular weight and polydispersity index of copolymer*

Molecular weight remaining ($M_R$) was calculated according to equation (3).

$$M_R=M_{n2}/M_{n1}*100\% \qquad \textbf{(3)}$$

$M_{n1}$: initial molecular weight; $M_{n2}$: molecuar weight after degradation
Polydispersity index (PI) of the specimens was analyzed by GPC.

## Results and discussion

*Water absorption*

The results of current research showed that[8] the hydrophilicity of materials is the key factor affecting the affinity between cell and biomaterials; and with the increase of hydrophilicity, the affinity would increase too.

**Table 1** Water absorption of different copolymers (25±0.1°C)

| Copolymer | Mn×$10^{-4}$ | Contact angle/° | $W_A$ /% |
|---|---|---|---|
| PLGA | 8.4 | 98 | 4 |
| P(LLA-BMD) | 8.6 | 74 | 21.3 |
| | 6.5 | 73 | 30.5 |

The results in table 1 showed that the water absorption rate of PLGA was only 4%, but P(LLA-BMD)'s reached to 20%. The water contact angel of P(LLA-BMD) was about 74°, and it was more hydrophilic than PLGA (the water contact angel: 98°). From the structure of these two copolymers, we can see that there are many continuous methyls in PLGA, and methyl is a hydrophobic group. Meanwhile, there is

–NH group in P(LLA-BMD) chain, which makes it more hydrophilic. And with the decrease of molecular weight of P(LLA-BMD), the copolymer chain was shortened. Therefore, the water molecules could enter more easily into the polymer chain, resulting in the increase of water absorption.

*Morphological features*

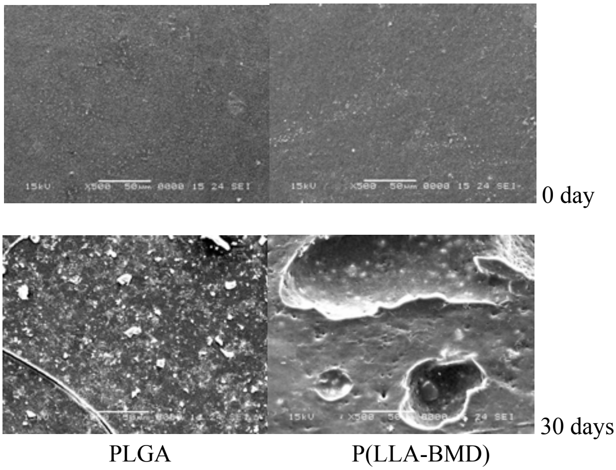

**Fig. 2** SEM of copolymer films (500×)(P(LLA-BMD): Mn=8.6*$10^4$; PLGA: Mn= 8.4*$10^4$)

After having degraded in PBS for 30 days, the films of P(LLA-BMD) and PLGA became slimsy. And the picture of SEM (×500) in fig.2 showed that there were so many changes having taken place on the surface of the films. The surface of PLGA became very rough, and there were many small fragments not dissolving in PBS. But there were a few holes instead of small fragments on the film of P(LLA-BMD). This might be resulting from the better hydrophilicity of this copolymer, so the residues of degradation were dissolved into PBS.

*Meight loss, molecular weight remaining and polydispersity index*

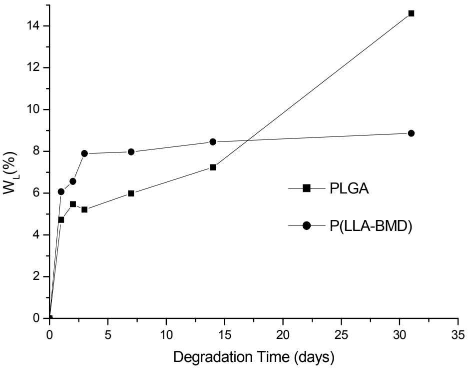

**Fig. 3** Meight Loss of copolymer (P(LLA-BMD): Mn=8.6*$10^4$; PLGA: Mn= 8.4*$10^4$)

To further study the degradation mechanism of P (LLA-BMD), we examined the weight loss and molecular weight remaining in PBS at 37°C for 0 to 90 days. Because of the breaking up of the copolymers after 30 days, we could not get the data of weight loss after 30 days.

It is known that the degradation mechanism of PLGA was bulk degradation.[9,10] So the weight loss and molecular weight remaining of this kind polymer had the same trends. The results in fig. 3 showed that the weight loss of P (LLA-BMD) was always higher than that of PLGA in the first 15 days. This contributed to the better hydrophilicity of P (LLA-BMD). After 15 days, the breaking up of PLGA film caused great weight loss. Many fragments were remained in the glass vessel, so the data was not the actual data.

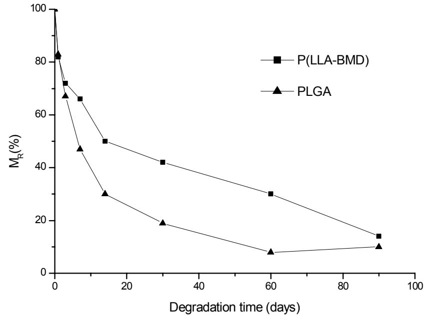

**Fig. 4** Molecular weight remaining of copolymers **(**P(LLA-BMD): Mn=8.6*$10^4$; PLGA:Mn= 8.4*$10^4$)

As is seen in fig.3 and fig.4, the two copolymers have the same trend of degradation (the molecular weight decreased as the weight decreased). One can conclude that the two copolymers have probably equivalent degradation behavior. This could also be confirmed by GPC analysis. The results in fig.5 also showed that with the increase of molecular weight the degradation tended to be slower. This also contributed to the better hydrophilicity of copolymer with lower molecular weight. The results in fig.6 showed that the evolution of the PI of P(LLA-BMD) and PLGA was also equivalent.

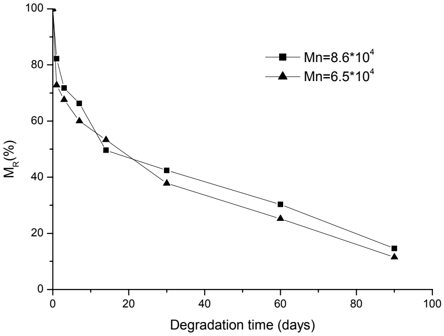

**Fig. 5** Molecular weight remaining of P(LLA-BMD) with different molecular weight

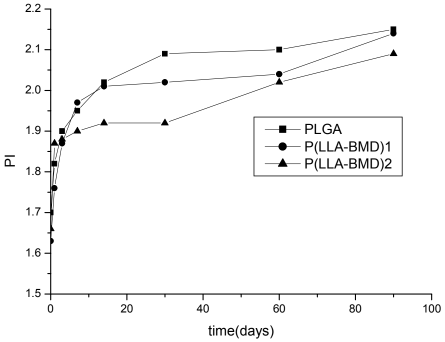

**Fig. 6**. Polydispersity index of polymers (P(LLA-BMD)1: Mn=8.6*$10^4$; P(LLA-BMD)2: Mn=6.5×$10^4$; PLGA:Mn= 8.4*$10^4$)

*Change of chemical structure*

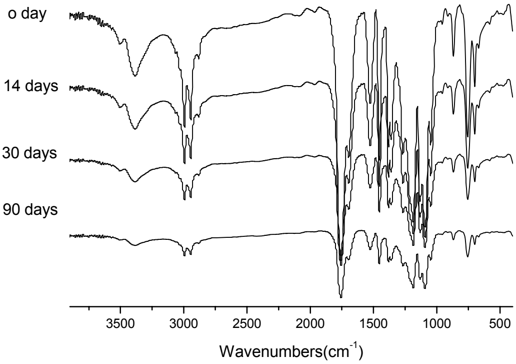

**Fig. 7** FT-IR of P(LLA-BMD) film after degradation (Mn=8.6*$10^4$)

The results in fig.7 showed that the chemical structure of P(LLA-BMD) did not change after having degraded in PBS in 90 days. To further study the mole composition of this copolymer, we analyzed it through $^1$H-NMR.

**Table 2** Mole composition of copolymers*

| Time (days) | $n_{LLA}/n_{GA}$ | $n_{LLA}/n_{BMD}$ |
|---|---|---|
| 0 | 80/20 | 83/17 |
| 30 | 83/17 | 83/17 |
| 60 | 90/10 | 84/16 |
| 90 | 91/09 | 85/15 |

*: determined By $^1$H-NMR spectroscopy ; P(LLA-BMD): Mn=8.6*$10^4$; PLGA:Mn= 8.4*$10^4$

From the results of table 2, the mole composition of GA in PLGA was decreasing from 20% to 9%, but the mole composition of BMD in P(LLA-BMD) had almost no change. This suggested that BMD in the copolymer chain was more stable, and this chain segment had almost the same degradation rate as L-LA chain segment in this copolymer.

## Conclusion

P(LLA-BMD) has better hydrophilicity than PLGA considering the results of water absorption. After 90 days PLGA and P(LLA-BMD) almost degraded completely in PBS at 37°C. Under the current experimental conditions the degradation mechanism of P(LLA-BMD) is probably bulk degradation, being the same as PLGA. And the chemical structure of P(LLA-BMD) did not change. The mole composition of P(LLA-BMD) was more stable than that of PLGA. Therefore, this biodegradable copolymer would have wider application prospects. Furthermore, the copolymer could bind with other substances through covalent bond in the side chain after hydrogenolysis. It could be expected to serve as a candidate material in the delivery of water-soluble drugs.

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## References

1. J. S. Chawla, M. M. Amiji (2002) Int. J. Pharm. 249 : 127
2. Q. Cai, J. Bei, S.Wang (2002) Polym. Adv. Technol. 13: 534
3. J. Jeong, J. Lee, K. Cho, J. Jeong, J. Lee, K. Cho (2003) J. Controlled Release 92: 249
4. H. Yoshimoto, Y. M. Shina, H. Teraia, J. P. Vacantia (2003) Biomaterials 24: 2077
5. M. R. Williamson, A. G. A. Coombes (2004) Biomaterials 25: 459
6. C. Tu, Q. Cai, J. Yang, Y.Wan, J. Bei, S.Wang (2003) Polym. Adv. Technol. 14: 565
7. Z. R. Tang, H. Huang, J. Rao, S. J. Cheng (2002) J. East China University of Science and Tecnology 28: 618
8. James M Anderson. (2001) Annual Review of Materials Research 31: 81
9. Achim Göpferich (1996) Biomaterials 17:103
10. Graham Swift (1998) Polymer degradation and stability 59:19