Direct Synthesis of Poly(D,L-lactic acid) by Melt Polycondensation and Its Application in Drug Delivery

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ABSTRACT: Starting from D,L-acid and $SnCl_2$ as catalyst, poly(D,L-lactic acid) (PDLLA) was directly synthesized by melt polycondensation. Under the appropriate conditions such as 0.5 wt % $SnCl_2$, 170–180°C, 70 Pa, and 10 h, the viscosity-average molecular weight (M_η) of PDLLA was 4100 Da. PDLLA produced by the most practical method was used as the drug-delivery material for erythromycin and ciprofloxacin. The optimal conditions for the preparation of erythromycin–poly(D,L-lactic acid)–microsphere (ERY–PDLLA–MS) for lung targeting was investigated, and further confirmed by good reappearance tests. DSC and SEM demonstrated that ERY–PDLLA–MS had good spherical shape. The release *in vitro* of ERY–PDLLA–MS was effective and the half-time ($T_{1/2}$) was 51.0 h. After 175 h, the accumulated release percentage was 80.0%. The test *in vivo* showed that ERY–PDLLA–MS was more easily distributed in rabbit lung tissue. When PDLLA was applied in an antibacterial ciprofloxacin drug-delivery microsphere (CIP–PDLLA–MS), CIP–PDLLA–MS was also characterized with DSC and SEM, and the release $T_{1/2}$ *in vitro* was 24.9 h. After 53.2 h, the accumulated release percentage reached 84.0%, which indicated that CIP–PDLLA–MS was advantageous to long-term release. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2143–2150, 2004

Key words: polycondensation; synthesis; drug delivery systems; poly(D,L-lactic acid) (PDLLA); microsphere

INTRODUCTION

Polylactic acid (PLA), a kind of aliphatic polyester, can be completely biodegraded into carbon dioxide and water, which makes it harmless and nontoxic to the environment. At the same time, as an important product from the biological material lactic acid, PLA's excellent biologic compatibility and biological resorbability allow it to be used extensively in medical fields, such as suture, bone fixation material, wounds dressing, ophthalmic implantation, and drug-delivery microsphere.[1]

PLA is often synthesized through the two-step method, in which lactide is synthesized from lactic acid and used in ring-opening polymerization. The lengthy process makes PLA rather expensive. Direct solution polycondensation of lactic acid has been reported recently, and the process is much simpler.[2,3] However, the azeotropic solvent used in polymerization is not easy to purge. Therefore, direct polycondensation without solvents has attracted increasing interest, and poly(L-lactic acid) (PLLA) has been successfully synthesized by simpler and cheaper melt polycondensation.[4–7]

Poly(D,L-lactic acid) (PDLLA) is amorphous, and there is no residual microcrystallinity after degradation *in vivo*. Therefore, when PLA is to be used as drug-delivery material, PDLLA is preferred. Thus, starting from D,L-lactic acid, PDLLA was synthesized by direct melt polycondensation in this study. The influences of synthetic conditions on PDLLA viscosity-average molecular weight are discussed. Its applications in drug-delivery microspheres of antibacterials such as erythromycin and ciprofloxacin are also reported.

EXPERIMENTAL

Measurements

^{1}H-NMR spectra were recorded with a Bruker DRX-400 NMR spectrometer (Bruker Instruments, Billerica, MA) with $CDCl_3$ as a solvent, and using TMS as an internal standard. Infrared spectra (IR) were obtained with an Analect RFX-65 infrared spectrometer (US). Viscosity-average molecular weight (M_η) of PDLLA was calculated from its intrinsic viscosity ($[\eta] = 5.45 \times 10^{-4} M_\eta^{0.73}$) with a Ubbelohde viscometer (Cannon-Ubbelohde, State College, PA) using $CHCl_3$ as a solvent.[8]

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TABLE I
Influences of Different Catalysts on M_η (170°C, 70 Pa, 10 h, Cat. 0.5 wt %)

Run	Catalyst	Dispersibility	Solubility	M_η (Da)
1	GeO_2	Good	Not	1000
2	TSA	Good	Good	2000
3	ZnO	Common	Wholly not	2100
4	$ZnCl_2$	Good	Partly dissolve	2400
5	SnC_2O_4	Good	Partly dissolve	1500
6	SnO_2	Common	Easily sediment	1800
7	SnO	Good	Wholly not	3200
8	$Sn(Oct)_2$	Common	In the end dissolve	3300
9	Sn	Bad	Agglomerate	3400
10	$SnCl_2$	Good	Good	4100

Differential scanning calorimetry (DSC) thermograms were recorded with a CDR-4P differential thermal analysis apparatus (Shanghai Exact Scientific Instrument, China) at a heating rate 10°C/min under nitrogen atmosphere. The content of erythromycin was determined with a JEOL UV-1601 ultraviolet spectrometer (JEOL, Tokyo, Japan). The morphology and size distributions of microspheres were characterized with a Hitachi S-510 scanning electron microscope (SEM; Hitachi, Ibaraki, Japan) and an Olympus optical photomicrography system (Olympus, Osaka, Japan).

Materials

Stannous octoate [$Sn(Oct)_2$] was purchased from Wako Pure Chemical Industries (Tokyo, Japan), and *p*-toluenesulfonic acid (TSA) was obtained from Nacalai Tesque Co. (Kyoto, Japan). Erythromycin was purchased from Zhejiang Pharmacy Corp., and erythromycin injection from the Dalian Meiluo pharmacy factory. Ciprofloxacin was purchased from the Guangzhou Nanxin pharmacy factory. Other chemical reagents, including stannous chloride and D,L-lactic acid, were purchased in Guangzhou. All these materials were used without further purification.

Direct melt polymerization synthesis of PDLLA and its structure characterization

After D,L-lactic acid was dehydrated for 6 h under 140°C and 4000 Pa in a three-neck flask equipped with mechanical stirrer and thermometer, a catalyst was added. Under a certain temperature (160–200°C) and absolute pressure 70 Pa, the melt polymerization was carried out for 5–20 h. When the reaction finished, purification after vacuum drying yielded PDLLA, a white powder product.

The structure of PDLLA was characterized with IR and ^{1}H-NMR. IR (KBr, cm^{-1}): 1757 (C═O); 1188, 1130, and 1092 (C—O—C); 2995 and 1454 (CH_3). ^{1}H-NMR (TMS, δ, ppm): 1.58 (3H, s, CH_3), 5.21 (1H, s, CH).

Applications of PDLLA as drug microspheres[9]

An antibacterial (either erythromycin or ciprofloxacin) and PDLLA were dissolved in organic menstruum, after which the solution was charged into the dispersing medium. When the suspended mixture system was uniform through stirring, the diffusing agent was added to form microspheres. By collecting the microspheres through filtration, and washing them with distilled water, erythromycin–PDLLA microsphere (ERY–PDLLA–MS), or ciprofloxacin–PDLLA microsphere (CIP–PDLLA–MS), was prepared after being dried in vacuum.

Characterization and properties of microspheres were by DSC and SEM. Definition of drug content: enveloping efficiency (%) = (weight of drug in the microsphere/total microsphere weight) × 100; drug load rate (%) = (drug content in microsphere/total use quality of drug) × 100. The release properties of drug microspheres *in vitro* and *in vivo*, and their stability, were also tested.

TABLE II
Factors and Levels of the Orthogonal Test for Preparation of ERY–PDLLA–MS

	Factor			
Level	*A*, PDLLA concentration (mg/mL)	*B*, erythromycin/PDLLA (weight ratio)	*C* gelatin concentration (%)	*D*, stirring rate (rpm)
1	20	0.2 : 1	0.2	1200
2	50	0.8 : 1	0.5	900
3	100	0.5 : 1	1.0	300

TABLE III
Results and Analysis of the Orthogonal Test for Preparation of ERY–PDLLA–MS

Run	*A*	*B*	*C*	*D*	Yield (%)	S_1 (μm)	S_2	S_3 (%)	S_4 (%)	$S = S_1 - S_2 + S_3 + S_4$
1	1	1	1	1	36.38	4.41	2.14	39.60	18.14	60.01
2	1	2	2	2	73.34	7.70	1.70	33.48	20.29	59.77
3	1	3	3	3	64.20	10.40	5.66	61.05	31.70	97.49
4	2	1	2	3	25.50	10.86	2.14	31.91	20.86	61.49
5	2	2	3	1	33.66	3.81	2.26	14.30	18.88	34.73
6	2	3	1	2	78.61	8.64	1.51	69.26	29.37	105.76
7	3	1	3	2	35.59	8.90	1.16	40.42	18.93	67.09
8	3	2	1	3	82.88	7.35	1.39	34.50	18.50	58.96
9	3	3	2	1	72.66	6.18	1.61	65.83	30.20	100.60
K_{1j}	217.27	188.59	224.73	195.34	Quality index of ERY–PDLLA–MS					
K_{2j}	201.98	153.46	221.86	232.62						
K_{3j}	226.65	303.85	199.31	217.94	Influences: B > D > C > A					
R	24.67	150.39	25.42	37.28	Conclusion: $A_3B_3C_1D_2$					
K_{1j}	173.92	97.47	197.87	144.76	Yield index of ERY–PDLLA–MS					
K_{2j}	137.77	189.88	171.50	187.54						
K_{3j}	191.13	215.47	133.45	172.58	Influences: B > C > A > D					
R	53.36	118.00	64.42	42.78	Conclusion: $A_3B_3C_1D_2$					

RESULTS AND DISCUSSION

Influence of melt polymerization on M_η of PDLLA

The influence of different catalysts on M_η is shown in Table I. The catalytic effect was related not only to kinds of metal in catalysts, but also to their dispersibility and solubility in the polymerization system. The stannic series of catalysts were better. For good dispersibility and solubility, $SnCl_2$ was best, and the corresponding M_η reached a maximum. When D,L-lactic acid was directly polycondensed under atmospheric pressure, the starting reaction temperature was 12°C lower than that without $SnCl_2$. Toluene sulfonic acid (TSA) also dispersed and dissolved well, but its catalysis was less than that with $SnCl_2$. Thus the inexpensive $SnCl_2$ was selected as the catalyst for direct melt polymerization.

Other factors influencing the M_η value of PDLLA, such as catalyst quantity, polymerization time, and temperature, were also investigated. As a major influencing factor, when the temperature was higher than 180°C, it lowered the yield of PDLLA for vacuum sublimation and partial carbonization, especially when the time was longer than 12 h. When the time was 10 h with 0.5 wt % $SnCl_2$, the highest M_η value of PDLLA was 4100 Da. Thus, the appropriate conditions were 0.5 wt % $SnCl_2$, temperature 170–180°C, absolute pressure 70 Pa, and time 10 h.

Compared with the direct solution polymerization method,[2,3] direct melt polymerization has good prospects in the development of PDLLA biodegradable material because of its simpler device and process with less consumption of chemical reagents, less time, more convenient purification, and lower cost. When the need for values of M_η of PDLLA that are not too high, the direct synthetic method by melt polymerization is especially practical. It has been reported that PDLLA, with molecular weight ranging between 3300 and 63,000 Da, could be used as a drug-delivery carrier.[10] PDLLA, directly synthesized by the above method, was also successfully applied in drug microspheres.

Preparation of ERY–PDLLA–MS

Erythromycin is a clinical medicine widely used for pneumonia caused by mycoplasma and legionnella.

TABLE IV
Reappearance of the Optimal Conditions for Preparation of ERY–PDLLA–MS

Run	Average particle diameter (μm)	Evenness	Enveloping efficiency (%)	Drug load rate (%)
1	10.83	1.363	62.61	24.21
2	11.23	1.186	63.04	23.21
3	10.80	1.421	64.10	24.01
4	11.01	1.315	63.93	24.43
5	11.18	1.511	63.41	24.21
6	10.91	1.396	64.17	24.89

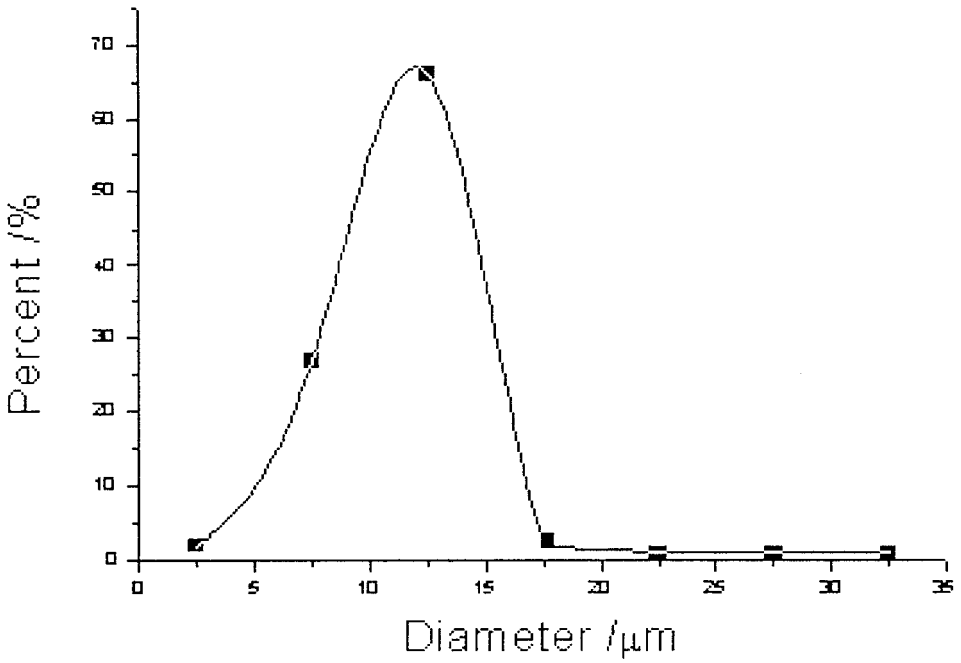

Figure 1 Particle size distribution of ERY–PDLLA–MS

However, its half-time is so short (only about 2 h) that the patients must take the medicine frequently. At the same time, as normally taken, including erythromycin injection, it is easily distributed all over the body and apt to induce harmful effects. To eliminate its side effects and to be more convenient, it is beneficial to use PDLLA as the drug-delivery material to make a lung-targeting erythromycin microsphere for controlled release.

The right organic menstruum was the key to forming PDLLA microspheres. Of chloroform and ethyl acetate with dichloromethane, the latter was better.

The viscosity of the dispersing medium had a significant effect on the particle diameter of PDLLA microspheres, and it depended on the dispersion degree of organic ingredient in the dispersing medium. Glycerin was an excellent dispersing medium, allowing even dispersal of the organic ingredient. Formation of

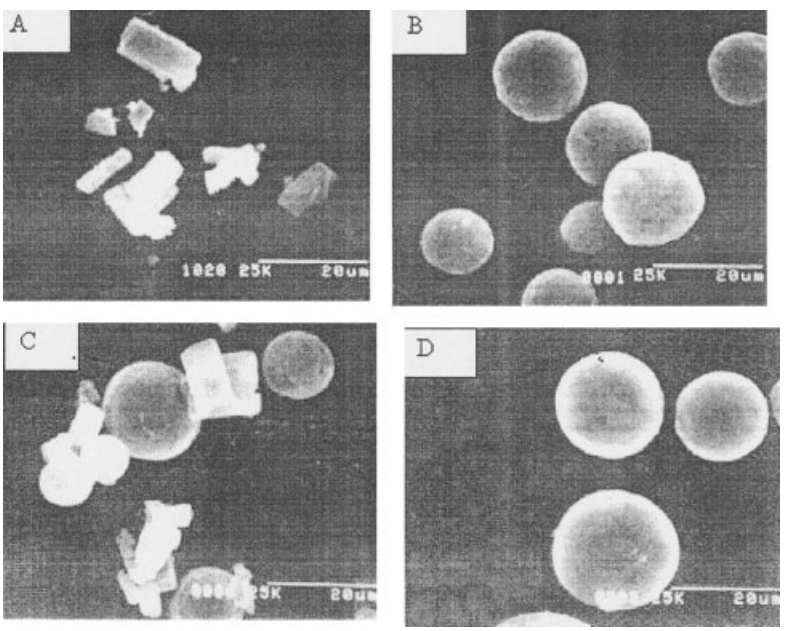

Figure 2 Morphology of erythromycin and different microspheres: (A) erythromycin; (B): PDLLA–MS; (C) mechanical mixture of erythromycin and PDLLA–MS; (D) ERY–PDLLA–MS.

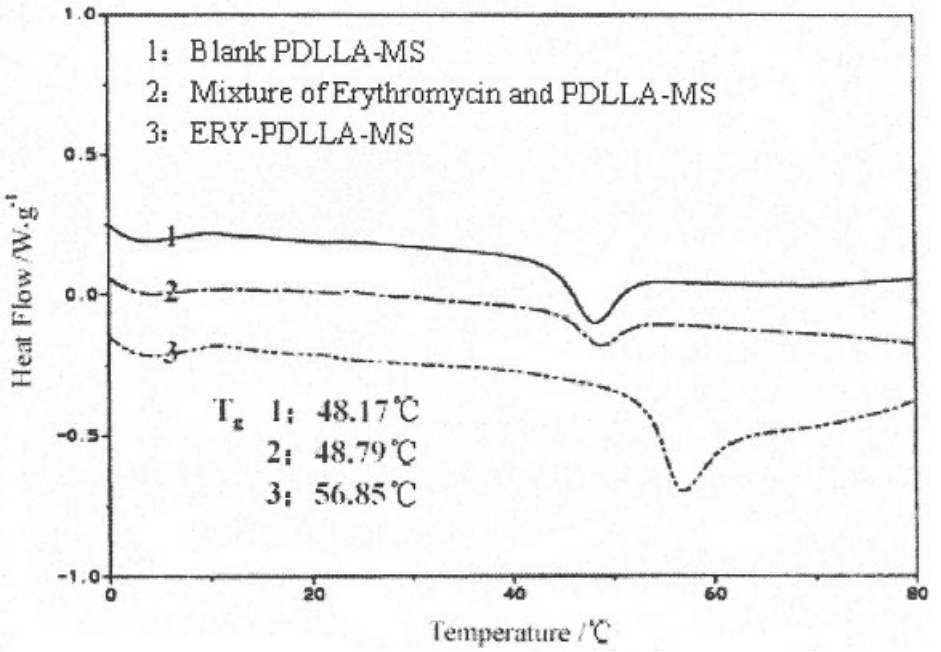

Figure 3 DSC of ERY–PDLLA–MS compared with other different microspheres.

PDLLA microspheres and control of particle diameter were easily obtained in glycerin. When the volume ratio was 3 : 40 (the organic ingredient to glycerin), a high drug loading rate and appropriate particle size were obtained.

Using gelatin water solution as a suitable diffusing agent to prevent microspheres from aggregating, when the volume ratio was 200 : 40 (gelatin to glycerin), well-dispersed microspheres were prepared.

Orthogonal tests (Table II and Table III) were used to examine the influences of some factors on the yield and quality of ERY–PDLLA–MS (S), such as drug load rate (S_4), enveloping efficiency (S_3), average particle diameter (S_1), and evenness (span length, S_2), where $S = S_1 - S_2 + S_3 + S_4$. These factors included the concentration of PDLLA solution (A, dichloromethane as solvent), the weight ratio of erythromycin to PDLLA (B), the concentration of gelatin water solution (C), and stirring rate (D).

Although different indices of ERY–PDLLA–MS gave a different influencing order, they led to the same conclusion (Table III): the optimal preparation conditions were dichloromethane/glycerin/gelatin water solution = 3 : 40 : 200 (volume ratio), PDLLA concentration 100 mg/mL, erythromycin/PDLLA = 0.5 : 1 (weight ratio), the concentration of gelatin water solution 0.2% (g/g), and stirring rate 900 rpm. The above result was further confirmed by the reappearance tests (Table IV), and thus the feasibility was good.

Characterization and properties of ERY–PDLLA–MS

To force a drug to remain in the lung mechanically through intravenous injection, the average particle size of a lung-targeting medicine should be between 7 and 20 μm.[11,12] Microscopic calculation gave the average particle size (11.18 μm) and size distribution of ERY–PDLLA–MS (Fig. 1). The sum of particles between 7 and 20 μm was more than 95%, which could meet the requirement for lung targeting.

From SEM micrographs of pure erythromycin [Fig. 2(A)], blank PDLLA microspheres [PDLLA–MS; Fig. 2(B)], the mechanical mixture of erythromycin and

TABLE V
Stability Test of ERY–PDLLA–MS

Temperature (°C)	Erythromycin load rate (%)		Microscopic observation 3 months later
	Original	3 months later	
4	24.21 ± 0.50	23.80 ± 0.48	Even distribution, round, and smooth shape
20–25	24.11 ± 0.72	23.69 ± 0.61	
37	24.69 ± 0.67	24.24 ± 0.80	

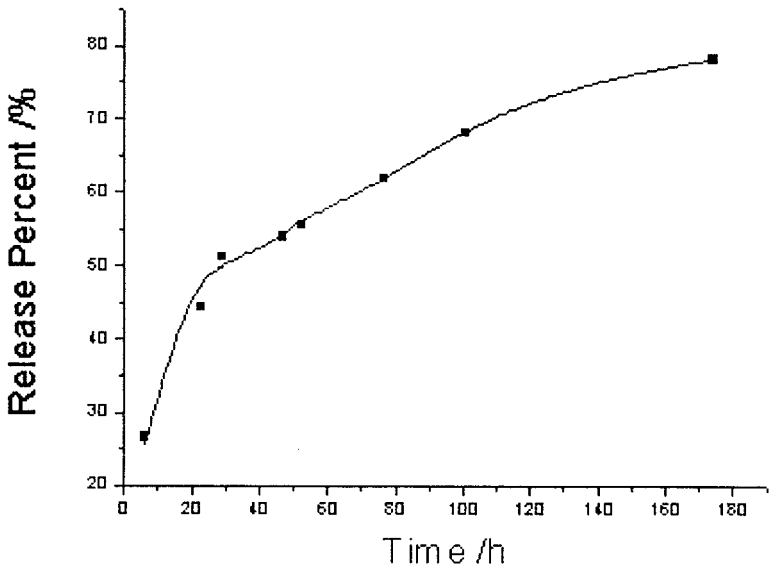

Figure 4 Release *in vitro* of ERY–PDLLA–MS.

PDLLA–MS [Fig. 2(C)], and ERY–PDLLA–MS [Fig. 2(D)], it may be observed that mechanically mixing was wholly disordered, but ERY–PDLLA–MS had a round and smooth shape without erythromycin crystals outside.

To verify the formation of the drug carrier, DSC was used to analyze and compare blank PDLLA–MS [Fig. 3(1)], the mechanical mixture [Fig. 3(2)], and ERY–PDLLA–MS [Fig. 3(3)]. Glass-transition temperature (T_g) data of different microspheres were 48.2, 48.8, and 56.9°C, respectively. The latter was 8°C higher than other two, which indicated that erythromycin was trapped in the PDLLA microsphere instead of attaching to its surface. Erythromycin is a macrolide antibiotic with some active hydroxyl groups, where hydrogen bonds form easily. As the result of formation of hydrogen bonds between PDLLA and erythromycin, the motility of the chain segments of PDLLA was decreased, and thus the T_g was increased.

To investigate the stability, samples of ERY–PDLLA–MS were sealed in small vials and stored under 75% relative humidity at different temperatures, such as 4°C, room temperature (20–25°C), and 37°C, respectively. After 3 months, their outer shape and erythromycin content were examined. The results (Table V) showed that almost no change occurred during the test period.

Release properties of ERY–PDLLA–MS *in vitro* and *in vivo*

Figure 4 shows the release half-time ($T_{1/2}$) of ERY–PDLLA–MS as 28 h. After 175 h, the accumulated release percentage was about 80%. Its Higuichi equation was $Q = 28.067 + 3.8515T_{1/2}$, $r = 0.9834$. The result proved that ERY–PDLLA–MS had a good sustained release effect, and the effective concentration of

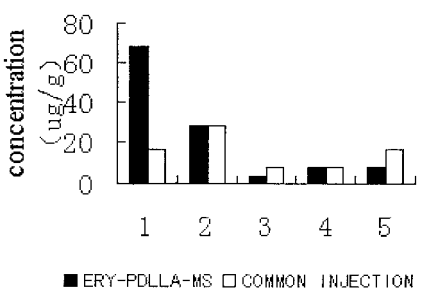

Figure 5 Concentration of erythromycin in different tissues: 1: lung; 2: liver; 3: heart; 4: spleen; 5: kidney.

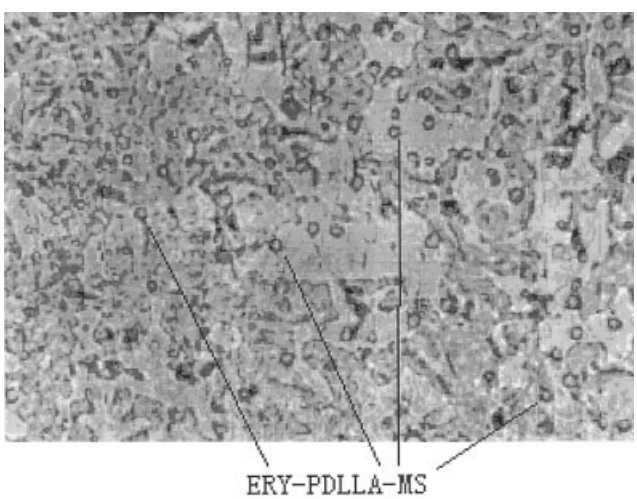

Figure 6 Optical photomicrography of rabbit lung tissue slice (×200).

erythromycin could be maintained for a relatively long time.

The aim of ERY–PDLLA–MS was lung targeting. Its corresponding tests *in vivo* were carried out on rabbit bodies. Figure 5 showed erythromycin distribution in different tissues 3 h after an intravenous injection into the rabbit's ear. The erythromycin concentration in lung tissues was evidently larger than that in liver, kidney, spleen, and heart. It was 4 times higher than that of common erythromycin injection in lung tissues. The result demonstrated that the lung-targeting effect is significant.

Optical photomicrography of lung tissue slice 6 h after the intravenous injection into the rabbit's ear showed that ERY–PDLLA–MS was mechanically trapped and evenly distributed in lung tissues (Fig. 6). It also proved that lung targeting was successful.

Application of PDLLA in CIP–PDLLA–MS

Similarly, the application of PDLLA as a ciprofloxacin microsphere carrier was also studied. As a kind of third-stage quinolone antibacterial, ciprofloxacin is a clinical medicine for many bacteria. Its common forms, including capsule, troche, dropping water for eye, and injection, are widely used for the prevention and cure of various microbial infections. However, the half-time of ciprofloxacin is too short (only 1.0–1.6 h) and its practical availability *in vivo* is about 52%. It is thus suitable to make into a controlled-release drug system.

At the same time, many pathogens can directly enter into a cell, but most antibacterials are stopped by the cell lysosome and cannot enter the inside of the cell to kill a pathogen. Therefore, to heighten the drug concentration and enhance the curative effect, it is necessary to directly bring the antibacterial into a cell by a ciprofloxacin microsphere. In the hope of curing repetitious peritonitis caused by peritoneal dialysis, CIP–PDLLA–MS was prepared by the same protocol as that for ERY–PDLLA–MS.

CIP–PDLLA–MS had good fluidity and was not sticky. The drug load rate of CIP–PDLLA–MS was 34.1 ± 0.51%, and the enveloping efficiency was 67.5 ± 0.58%. To obtain a good curative effect, and to eliminate side effects on the body, the average particle size should be between 250 and 425 μm. The microscopic calculation showed that the practical average particle size was 280.80 μm, and the sum of particles between 250 and 425 μm was more than 92% (Table VI), which should satisfy the requirement to cure repetitious peritonitis.

TABLE VI
Diameters of CIP–PDLLA–MS and Their Distribution

Diameter range (μm)	Average diameter (μm)	Distribution (%)
0–100	50	2
101–200	150	6
201–300	250	21
301–400	350	59
401–450	425	12

TABLE VII
Stability Test of CIP–PDLLA–MS

Temperature (°C)	Ciprofloxacin load rate (%)	
	Original	One month later
4	34.10 ± 0.50	34.11 ± 0.72
25	33.80 ± 0.48	33.49 ± 0.61

SEM micrographs of CIP–PDLLA–MS showed that the shape was approximately round, without quadrate ciprofloxacin crystals outside. The particle diameter of CIP–PDLLA–MS was also even. Through DSC analysis of CIP–PDLLA–MS, T_g data of different microspheres were 52.5°C (blank PDLLA–MS), 52.7°C (mechanical mixture of ciprofloxacin and PDLLA–MS), and 58.3°C (drug carrier CIP–PDLLA–MS). The latter was nearly 6°C higher than other two, which indicated that ciprofloxacin was trapped in the PDLLA microsphere instead of attaching to its surface.

CIP–PDLLA–MS had good stability. After a 1-month test period, the drug content and the outer shape were almost identical (Table VII).

Figure 7 shows that the release half-time ($T_{1/2}$) of CIP–PDLLA–MS was 24.9 h, and the accumulated release percentage was about 84.0% after 53.2 h. Its Higuichi equation was $Q = -0.0043 + 0.0039T_{1/2}$, $r = 0.994$. The result demonstrated that CIP–PDLLA–MS had an obvious controlled release effect and its release accorded with the characteristics of a long-efficiency medicine form.

CONCLUSIONS

1. Starting from D,L-lactic acid, PDLLA could be synthesized by direct melt polycondensation. By choosing the appropriate conditions (0.5 wt

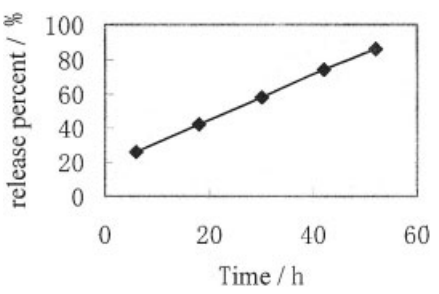

Figure 7 Release *in vitro* of CIP–PDLLA–MS.

% $SnCl_2$ as catalyst, absolute pressure 70 Pa, temperature 170–180°C, and time 10 h), the highest M_η of PDLLA could be achieved, 4100 Da.
2. Using PDLLA produced as above as the delivery carrier of the antibacterials erythromycin and ciprofloxacin, ERY–PDLLA–MS and CIP–PDLLA–MS were prepared.

ERY–PDLLA–MS had good spherical shape, with a half-time ($T_{1/2}$) of 51.0 h. After 175 h, the accumulated release percentage was 80.0%. The test *in vivo* showed that ERY–PDLLA–MS was more easily distributed in rabbit lung tissue.

The release $T_{1/2}$ *in vitro* of CIP–PDLLA–MS was 24.9 h. After 53.2 h, the accumulated release percentage was 84.0%. The release *in vitro* of CIP–PDLLA–MS showed a significant sustained release.

3. The successful synthesis and applications proved that usable PDLLA could be more easily obtained by the direct melt polycondensation of the cheaper monomer D,L-lactic acid.
4. The process of the direct melt polycondensation method was simpler, not only than that of the two-step method of D,L-lactide ring-opening polymerization, but also than that of the direct solution polycondensation method. The amounts of chemical material and reagent were also significantly smaller. Thus the direct melt polycondensation method is superior to the other two methods to some extent.

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References

1. Kricheldorf H. R. chemophere 2001, 43, 49.
2. Ajioka M.; Enomoto K.; Suziki K.; Yamaguchi A. Bull. Chem Soc Jpn 1995, 68, 2125.
3. Mai H. Z.; Zhao Y. M.; Wang J Polymer Preprints 2001′42(2), 366.
4. Moon S. I.; Lee C. W.; Miyamoto M., et al. Polym. Sci., Part A: Polym. Chem 2000, 38, 1673.
5. Moon S. I.; Lee C. W.; Taniguchi I., et al. Polymer 2001, 42, 5059.
6. Mai H. Z.; Zhao Y. M.; Wang Z. Y., et al. Polymer Preprints 2002′43(1), 526.
7. Wang Z. Y.; Zhao Y. M.; Mai H. Z., et al. Polymer Preprints 2002′43(1), 528.
8. Schindler A.; Harper D. J Polym Sci, Polym. Chem Ed 1979, 17, 2593.
9. Yang Q.; Owusu A. G. Drug Dev. Ind Pharm 2000, 26, 61.
10. Delgado A.; Evora C.; Dabres M. Int J Pharm 1998, 166, 223.
11. Kanke M.; Simmous G. H.; Weiss D. L., et al. J Pharm Sci 1980, 69, 755.
12. Pande S.; Vyas S. P.; Dixit V. K. J Microencapsul 1991, 8, 87.