Characterization of Poly(D,L-lactic acid) Synthesized by Direct Melt Polymerization and Its Application in Chinese Traditional Medicine Compound Prescription Microspheres

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ABSTRACT: From D,L-lactic acid and with 0.5 wt % stannous chloride ($SnCl_2$) as a catalyst under an absolute pressure of 70 Pa and at 165–170°C, poly(D,L-lactic acid) (PDLLA) with an intrinsic viscosity of 0.2302 dL/g was synthesized after 10 h of melt polycondensation. This directly produced PDLLA was systematically characterized with gel permeation chromatography, Fourier transform infrared, ^{1}H-NMR, differential scanning calorimetry, and X-ray diffraction. The weight-average molecular weight of PDLLA was 17,800 Da, and the weight-average molecular weight/number-average molecular weight ratio was 1.25. It was partly crystalline, and its crystallinity was 20.8%. When first applied to the Chinese traditional medicine compound prescription Osteitis no. 1 PDLLA microsphere (G-PDLLA-MS), the optimal conditions for the extraction of the effective components in three raw herbs (*Herba epimedii*, *Fructus psorakae*, and *Angelicae*) and for the preparation of G-PDLLA-MS were determined with orthogonal testing. After the extractant was obtained by ethanol reflux, G-PDLLA-MS was prepared with a gelatin concentration of 8%, an inner water phase/oil phase volume ratio of 1.5:1, a stirring rate of dispersion of 1000 rpm, and a PDLLA concentration of 20%. Differential scanning calorimetry and scanning electron microscopy proved that G-PDLLA-MS had a good spherical shape. The practical average particle size of G-PDLLA-MS was 8.59 μm, and the particle sum between 2 and 12 μm was greater than 92%; this meets the requirement for an arthritis cure. J Appl Polym Sci 97: 195–200, 2005

Key words: polycondensation; synthesis; biodegradable; drug delivery systems

INTRODUCTION

As an important biodegradable material, poly(lactic acid) (PLA) has many particular properties, such as good biocompatibility and safety *in vivo*, that make it extensively used in medical applications, including sutures, bone fixation materials, wound dressings, and drug-delivery microspheres.[1] However, PLA is often synthesized by the ring-opening polymerization of lactide, and this lengthy process makes it rather expensive. To reduce the cost of PLA and simplify its synthetic process, more and more importance has been attached to the direct polymerization of lactic acid,[2–6] especially direct melt polycondensation, which is more practical.[3,7–17]

Poly(D,L-lactic acid) (PDLLA) is more suitable than poly(L-lactic acid) (PLLA) as a drug-delivery material.[17] However, there have been few publications reporting the systematic characterization of directly produced PDLLA. Therefore, from D,L-lactic acid (D,L-LA), PDLLA was synthesized by direct melt polycondensation and characterized in detail in this study.

Osteoarthritis (OA) is one of the most frequent orthopedic clinical diseases and also one of the most familiar arthropathies in the world. With the aging of the population, the incidence of OA is becoming higher and higher. OA can be cured by Western medicine or Chinese traditional medicine. The former has a quick curative effect with side effects, and the disease is likely to recur. However, the latter can eradicate OA satisfactorily, especially with Chinese traditional medicine compound prescription Osteitis no. 1.

Osteitis no. 1 comes from long-term and abundant clinic practice and is made up of three Chinese traditional medicines: *Herba epimedii*, *Fructus psorakae*, and

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Angelicae. Normally, the forms of Chinese traditional medicines are decoctions for drinking and pastes to be used on the skin. The former are absorbed by the stomach, and the effective component may be distributed all over the body and lead to side effects. The latter must take action after the absorption of the drug through the skin. Therefore, their practical efficacies are both lessened to a degree, and the ill must take medicine frequently. At the same time, decocting Chinese traditional medicines is rather tiresome for the ill.

To eliminate the need of taking a compound prescription medicine frequently, to make it more convenient for the ill to take a medicine, and to further improve the curative effects with fewer side effects, using PDLLA as a drug-delivery material to make microspheres is a good idea. When the Osteitis no. 1 PDLLA microsphere (G-PDLLA-MS) is injected into the knee-joint cavity, it can highly selectively act on knee OA with the degradation of PDLLA *in vivo*. Because the concentration of the drug is higher than that of the normal form in the inflammation area, the action of the novel dose is stronger and continues for a longer time. Therefore, the first application of PDLLA to delivery microspheres of Osteitis no. 1 is reported in this article.

EXPERIMENTAL

Measurements

^{1}H-NMR spectra were recorded with a DRX-400 NMR spectrometer (Bruker Instruments, Billerica, MA) with $CDCl_3$ as the solvent and internal standard. IR spectra were obtained with a Bruker Vector 33 Fourier transform infrared (FTIR) spectrometer. Differential scanning calorimetry (DSC) thermograms were recorded with an American PerkinElmer DSC7 thermal analysis apparatus (PerkinElmer, Norwalk, CT) at a heating rate of 10°C/min under an argon atmosphere. With a wavelength of 1.5406×10^{-10} m and a scanning scope of $2\theta = 1–40°$, a Japan Rigaku D/Max 1200X X-ray diffractometer (Rigaku, Tokyo, Japan) was used to investigate the crystallinity of PDLLA.

The intrinsic viscosity ([η]) of PDLLA was determined with an Ubbelohde viscometer (Cannon-Ubbelohde, State College, PA) with $CHCl_3$ as the solvent at 25°C.[17] The weight-average molecular weight (M_w) and molecular weight distribution were determined with a Waters 515 high-performance liquid chromatograph with tetrahydrofuran as the solvent and polystyrene as the reference at a flow velocity of 1 mL/min and at 35°C.

With the concentration of *F. Psorakae* (one ingredient of Osteitis no. 1) as the detection index, the concentration of the drug was determined with a UV-1601 ultraviolet spectrometer (JOEL, Tokyo, Japan). The morphology and size distributions of the microspheres were characterized with an S-510 scanning electron microscope (Hitachi, Ibaraki, Japan) and an optical photomicrography system (Olympus, Osaka, Japan).

Materials

The raw herbs used in Osteitis no. 1 (*H. epimedii*, *F. Psorakae*, and *Angelicae*) were purchased from Guangzhou Medicinal Materials Co. (Guangzhou, China). D,L-LA and $SnCl_2$ were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China) and Guangzhou Donghong Chemical Factory (Guangzhou, China), respectively. Other chemical reagents were also purchased in Guangzhou. All these materials were directly used without further purification.

Direct melt polymerization synthesis of PDLLA

After D,L-LA was dehydrated for 6 h at 140°C and 4000 Pa in a three-necked flask equipped with a mechanical stirrer and a thermometer, the catalyst $SnCl_2$ (0.5 wt %) was added. At a certain temperature (165–170°C) and an absolute pressure of 70 Pa, the direct melt polymerization was carried out. After 10 h of reaction, purification and drying *in vacuo* yielded PDLLA as a white powder.[17]

Extraction of the effective component in Osteitis no. 1

Before the preparation of G-PDLLA-MS, the effective and successful extraction of Osteitis no. 1 was first investigated. For the extraction of the effective components in Chinese traditional medicine, three main methods—refluxing, seeping, and ultrasound stirring—have been reported in the literature. Therefore, for Osteitis no. 1, *H. epimedii*, *F. psorakae*, and *Angelicae* were tested with the three extraction methods. The factors influencing the extraction efficiency, including the solvent ethanol weight (times the raw herb weight), the number of extractions, the ethanol dipping time, and the ethanol concentration (Table I), were also examined through orthogonal tests.[18–20]

With the extraction of *F. Psorakae* (one ingredient of Osteitis no. 1) as an example, both the refluxing method and ultrasound stirring method were designed as shown in Table I. The results showed that the optimal extraction conditions for the different extraction methods were as follows: 70% ethanol (8 times the weight of *F. Psorakae*), three extractions, and 60 min each time. However, the extractant qualities of the effective components were different. The quality via refluxing was 33.60 mg/g, and the quality via ultrasound stirring was 19.91 mg/g. The former was obviously higher than the latter.

Similarly, in other experiments used to compare different methods and different raw herbs, refluxing

TABLE I
Factors and Levels of the Orthogonal Test for the Extraction of *F. psorakae*

	Factors			
Level	Solvent ethanol weight (× weight of *F. Psorakae*)	Extraction time	Ethanol dipping time (min)	Ethanol concentration (%)
1	4	1	40	70
2	6	2	50	60
3	8	3	60	50

was better than the other methods. Thus, we concluded that refluxing in ethanol was best. When it was used to extract the effective components in Osteitis no. 1 (*H. epimedii*, *F. psorakae*, and *Angelicae*), the following appropriate conditions were found: 80% ethanol as the solvent, two refluxings of the mixed raw herbs, and 45 min each time.

Therefore, the whole extraction process for Osteitis no. 1 was as follows. *H. epimedii*, *F. psorakae*, and *Angelicae* were added together in equal amounts. With 80% ethanol as the solvent, the raw herb mixture was dipped and refluxed for 45 min. After two refluxings, the decoction of the compound prescription was filtered, and the removal of the solvent ethanol produced a dense cream. This extractant was the effective component of Osteitis no. 1.

Application of PDLLA as drug microspheres

With the extractant as the drug and with the same method used for antibacterial (erythromycin and ciprofloxacin) PDLLA microspheres,[17] G-PDLLA-MS was prepared with the emulsification–evaporation method.[17,21] Then, G-PDLLA-MS was characterized with DSC and scanning electron microscopy (SEM), and its average particle size and particle size distribution were detected through microscopy calculations.

RESULTS AND DISCUSSION

Structure and properties of PDLLA synthesized by direct melt polycondensation

The structure of PDLLA was characterized with FTIR and ^{1}H-NMR.

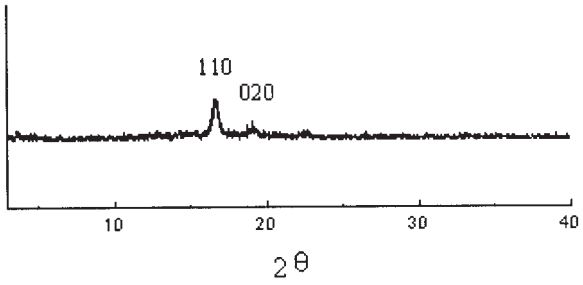

Figure 1 XRD spectrum of PDLLA synthesized by direct melt polycondensation.

IR (KBr, cm^{-1}): 1759 (C═O, strong), 1213, 1187, 1134, 1093 (C—O—C, strong), 2998, 2947, 1457, 1386, 1362 (CH_3, CH), 3505 (terminal OH, weak). ^{1}H-NMR ($CDCl_3$ as the solvent, δ = 7.28 ppm as the internal standard, δ, ppm): 1.59 (3H, *d*, CH_3), 5.18 (1H, *q*, CH).

Therefore, the data showed that the structure of PDLLA synthesized by the direct melt polycondensation was correct.

Normally, PDLLA with a molecular weight of 3300–63,000 Da can be used as a drug-delivery carrier.[17,22] The molecular weight of PDLLA cannot be too low, but too high a molecular weight is not advantageous for the formation of microspheres of an appropriate size. Therefore, despite the successful applications in drug microspheres of PDLLA synthesized by direct melt polymerization,[17] M_w and the molecular weight distribution of PDLLA were further investigated with gel permeation chromatography (GPC).

The GPC results showed that, when [η] of PDLLA was 0.2302 dL/g {according to the formula,[23] $[\eta] = 5.45 \times 10^{-4} M_\eta^{0.73}$, the corresponding viscosity-average molecular weight (M_η) was 4000 Da}, M_w was 17,800 Da, and the number-average molecular weigh (M_n) was 14,200 Da. The polydispersity index (M_w/M_n) was 1.25. This proved that directly produced PDLLA had a very sharp molecular weight distribution, and this could be advantageous for the preparation of microspheres. Therefore, direct melt polymerization is very practical and has good potential for the development of biodegradable PDLLA as a drug microsphere carrier because of its simpler process and lower cost.[17]

DSC data for PDLLA synthesized by direct melt polycondensation showed that the glass-transition temperature (T_g) was 54.57°C, and the melting temperature (T_m) was 120.02°C. The DSC thermogram

TABLE II
XRD Results of PDLLA Synthesized by Direct Melt Polymerization

2θ			Crystal dimension (10^{-10} m)	
110 reflection (°)	020 reflection (°)	Crystallinity (%)	d_{110}	d_{020}
16.7	19.1	20.8	154.4	83.9

TABLE III
Factors and Levels of the Orthogonal Test for the Preparation of G-PDLLA-MS

	Factors			
Level	Glutin concentration (%)	Inner water phase/oil phase (volume ratio or drug feed ratio)	Stirring rate (rpm)	PDLLA concentration (%)
1	8	1.0:1	800	10
2	10	1.5:1	1000	15
3	12	2.0:1	1200	20

showed that, when the temperature increased to 283.55°C, the thermal decomposition of PDLLA was most obvious, and so the decomposition temperature of PDLLA was 283.55°C.

In addition to T_m, the melting heat of PDLLA was detected, and it was 17.12 J/g. Therefore, although it has usually been regarded as an amorphous polymer, PDLLA synthesized by direct melt polymerization may be partly crystalline.

This conclusion, that PDLLA directly produced from D,L-LA may have a certain crystallinity, was further proved by X-ray diffraction (XRD; Fig. 1). The corresponding data are shown in Table II. PDLLA had two diffraction peaks, one at $2\theta = 16.7°$ due to the 110 reflection and one at $2\theta = 19.1°$ due to the 020 reflection, and both diffraction positions were very close to those of PLLA synthesized by the ring-opening polymerization of L-lactide.[24]

Preparation of G-PDLLA-MS

The right organic menstruum was the key to the formation of the PDLLA microsphere. Of chloroform and ethyl acetate with dichloromethane, the latter was better. Therefore, a dichloromethane solution of PDLLA was selected as the oil phase, and a glutin water solution was selected as the inner water phase.

The viscosity of the dispersing medium had a great effect on the particle diameter of the PDLLA microspheres, and a 2% poly(vinyl alcohol) water solution containing the Osteitis no. 1 extractant was a wonderful dispersing medium. When it was selected as the outer water phase, a large drug loading rate and an appropriate particle size could be obtained. At the same time, with a 1% NaCl water solution as the pervasion medium, satisfactory microspheres could be prepared.

Orthogonal tests (Tables III and IV) were used to examine the influences of some important factors on the preparation of G-PDLLA-MS, and its quality was evaluated with the average particle diameter (S_1) and especially the drug concentration (S_2). These factors included the concentration of the gelatin water solution, the inner water phase/oil phase ratio (volume ratio or drug feed ratio), the stirring rate, and the concentration of the PDLLA dichloromethane solution.

The optimal preparation conditions were a gelatin concentration of 8%, an inner water phase/oil phase volume ratio of 1.5:1, a stirring rate of dispersion of

TABLE IV
Results and Analysis of the Orthogonal Test for the Preparation of G-PDLLA-MS

Run	*A* (%)	*B*	*C* (rpm)	*D* (%)	S_1 (μm)	S_2 (μg/mL)
1	1	1	1	1	7.86	100.5282
2	1	2	2	2	8.62	113.2042
3	1	3	3	3	9.34	96.1268
4	2	1	2	3	9.31	111.9718
5	2	2	3	1	8.86	90.1409
6	2	3	1	2	9.49	87.1479
7	3	1	3	2	8.42	39.0845
8	3	2	1	3	8.41	76.0563
9	3	3	2	1	7.22	55.1056
K_{1j}	309.859	251.585	263.732	245.775		
K_{2j}	289.261	279.401	280.282	239.437	Influences: $A > C > D > B$	
K_{3j}	170.246	238.380	225.352	284.155	Conclusion: $A_1B_2C_2D_3$	
$R_j/3$	46.538	13.674	18.310	14.906		

A = concentration of gelatin water solution; *B* = inner water phase/oil phase volume ratio or drug feed ratio; *C* = stirring rate; *D* = PDLLA concentration.

1000 rpm, and a PDLLA concentration of 20% (Table IV). This conclusion was further confirmed by the reappearance tests, so the feasibility was good. According to the best preparation combination, G-PDLLA-MS was prepared and further characterized.

Characterization of G-PDLLA-MS

Microscopic calculations yielded a G-PDLLA-MS average particle size of 8.59 μm, and the sum of the particles between 1 and 12 μm was greater than 92%. The particle diameter of a drug injected into the body through the skin is ordinarily less than 50 μm. Thus, G-PDLLA-MS could meet the requirement for an arthritis cure through direct injection.

Observed under an optical microscope and a scanning electron microscope, G-PDLLA-MS had a round and smooth shape (Fig. 2). It was approximately evenly distributed, and little was conglutinated. In other words, G-PDLLA-MS had good fluidity and was not sticky.

To verify the formation of the drug carrier, we used DSC to analyze and compare blank PDLLA-MS [Fig. 3(1)], a mechanical mixture of the blank and drug [Fig. 3(2)], and G-PDLLA-MS [Fig. 3(3)]. The T_g values for the different microspheres were 42.74, 43.39, and 45.01°C, respectively. The last was 2.17°C higher than that of the blank and 1.62°C higher than that of the mechanical mixture. This indicated that the drug was trapped in the PDLLA microsphere rather than attached to its surface.

Most of the effective components in Osteitis no. 1 are polar and water-soluble. When they were trapped by PDLLA, a hydrogen bond easily formed between the carrier and drug. Therefore, the motility of the chain segments of PDLLA decreased, and T_g of G-PDLLA-MS increased. At the same time, different DSC thermograms had melting peaks near 120–122°C, and they were all caused by PDLLA.

CONCLUSIONS

From D,L-LA, with 0.5 wt % $SnCl_2$ as a catalyst, under an absolute pressure 70 Pa, and at 165–170°C, PDLLA with an [η] value of 0.2302 dL/g could be directly synthesized after 10 h of melt polycondensation. GPC data showed that M_w was 17,800 Da and M_w/M_n was 1.25. DSC and XRD testing showed that PDLLA directly produced from D,L-LA was partly crystalline, and its crystallinity was 20.8%.

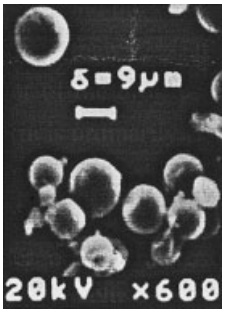

Figure 2 Morphology of G-PDLLA-MS by SEM.

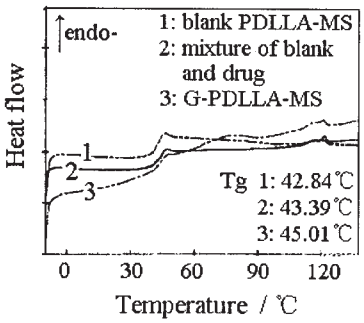

Figure 3 DSC of G-PDLLA-MS and other different microspheres.

G-PDLLA-MS was obtained under optimal preparation conditions: a gelatin concentration of 8%, an inner water phase/oil phase volume ratio of 1.5:1, a stirring rate of dispersion of 1000 rpm, and a PDLLA concentration of 20%.

DSC and SEM demonstrated that G-PDLLA-MS had a good spherical shape. The average particle size of G-PDLLA-MS was 8.59 μm, and the sum of the particles between 1 and 12 μm was greater than 92%. This could meet the requirement for an arthritis cure through direct injection and make the treatment more convenient and effective.

This successful synthesis, characterization, and application have proved that PDLLA can be more easily obtained by the simpler direct melt polycondensation of the cheaper monomer D,L-LA. This is advantageous for further research on Chinese traditional medicine and water-soluble drug-delivery systems.

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