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Preparation and evaluation of rotigotine-loaded implant for the treatment of Parkinson's disease and its evolution study

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KEYWORDS

Rotigotine-loaded implant;
Sustained release;
Parkinson's disease;
Continuous dopaminergic stimulation

Abstract *Purpose:* To develop rotigotine-loaded implants (RI) to achieve continuous release of rotigotine for long duration for the treatment of Parkinson's disease (PD).

Methods: RI was prepared by hot-melt extrusion method using poly (lactide-co-glycolide) (PLGA) as the matrix. *In vitro* drug release was optimized by drug loading, melting temperature during preparing process and additives. The surface and internal morphology of RI was imaged by SEM. The in vivo release profile of RI was investigated on rat.

Results: RI prepared with PLGA 7525 5A showed sustained release of 40 days while suffering a lag phase, which was significantly shortened by blending 5050 2A and mannitol in the matrix. RI prepared by 7525 5A/5050 2A/mannitol = 55:10:5 (rotigotine 30%) showed a 40-day sustained release in vivo with no lag phase. The drug release from RI was also affected by drug loading and melting temperature probably due to the drug state existed in the implant. The evolution of implants during release process was correlated well with the drug release kinetics.

Conclusion: RI could achieve sustained drug release for 40 days which could supply an alternative of continuous dopaminergic stimulation (CDS) for the treatment of PD.

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1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide which results primarily from the death of dopaminergic neurons in the substantia nigra (William and Przedborski, 2003) and it affects about 1–1.5% of the population over 60 years of age (De Lau and Breteler,

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2006). Levodopa continues to be gold standard for the symptomatic treatment of PD (Olanow et al., 2006a–c). However, after a few years of treatment with levodopa most parkinsonian patients develop motor complications, notably levodopa-induced dyskinesias (LID), which are difficult to control and significantly impair the quality of life. The appearance of LID may be related with the discontinuous stimulation toward dopamine receptor (Olanow et al., 2006a–c; Nyholm, 2007). Thus a new therapeutic concept of continuous dopaminergic stimulation (CDS) was proposed for the treatment of PD (Olanow et al., 2006a–c). Theoretically, CDS can continuously provide dopamine or a dopamine agonist to striatal dopamine receptors which should mimic the continuous stimulation of dopaminergic receptors in the normal manner to benefit the PD patients (Olanow et al., 2000, 2006a–c; Nutt, 2007). L-DOPA and dopamine agonists have inspired the therapeutic concept of CDS, which has been developed and received considerable attention for treatment of PD (Olanow et al., 2000, 2006a–c; Nutt, 2007; Nutt et al., 2000).

Rotigotine is a non-ergoline agonist of dopamine D3/D2/D1 receptor indicated for the treatment of PD. It was developed as transdermal patch, Neupro®, to provide 24-h continuous drug levels which could improve efficacy and reduce motor complications. However, there is many side effects with skin patches, eg the different absorption rate of drug through the different part of skin and the skin of different person and the skin irritation after long-term application (Sujith and Lane, 2009). Therefore, we have developed rotigotine-loaded microspheres in our previous study which could supply a 14-day sustained drug release (Wang et al., 2012). As PD patients require long-term treatment, it is necessary to develop a much longer sustained release preparation of rotigotine to achieve CDS for the treatment of PD and to improve the compliance of the PD patients significantly.

Polymeric implantable drug delivery systems have shown significant potential for systemic delivery of various therapeutic drugs (Jain et al., 2005) and proteins (Hafeman et al., 2008) at a controlled rate over the past few decades. Several polymeric implants, such as goserelin (Zoladex®), leuprolide (Viadur®) etc. (Furr and Hutchinson, 1991), are approved by the FDA. Due to their slow-release kinetics, implants can provide continuous drug release ranging from months to >1 year, which improves the patient compliance significantly (Dashand and Cudworth, 1998). Hot-melt extrusion has been developed as a new preparation technique for the implant in the present time, which has some advantages, including ease of use and lack of residual solvents, decreased costs and environmental hazards (Forster et al., 2001; Crowley et al., 2004).

Thus, the present study was designed to develop rotigotine-loaded implant to achieve much longer sustained release of rotigotine for the treatment of PD. The factors affecting drug release from the implant were investigated to obtain longer sustained release period. *In vivo* release behavior was also studied on rats.

2. Materials and methods

2.1. Materials and animals

PLGA 7525 5A (lactide/glycolide ratio, 75/25; Mw, 72,000), and PLGA 5050 2A (lactide/glycolide ratio, 50/50; Mw, 18,000) were purchased from Lakeshore Biomaterials, USA. Rotigotine was obtained from Shandong Luye Pharmaceuticals (Yantai, China). HPLC grade acetonitrile and methanol were supplied by Merck Specialities Private Ltd. (USA). All other reagents used in this study were analytical grade.

Male Sprague–Dawley rats (200–250 g) used in the pharmacokinetics study were obtained from the Experimental Animal Center of Shandong Luye Pharmaceutical Co., Ltd. (Yantai, China). All of the experiments were carried out in accordance with the 'Principles of Laboratory Animal Care' (NIH publication No. 85-23, revised 1985) and the Experimental Animal Research Committee at Yantai University.

2.2. Preparation of rotigotine-loaded implants (RI)

Rotigotine-loaded implants were prepared by hot-melt extrusion method. The drug and PLGA were weighed at the selected proportions as listed in Table 1 and gently ground in a oscillating miller (MM400 Retsch GmbH, Germany) for 3 min (25 Hz s^{-1}) to achieve uniformity of powder. The extrusion process was performed using a twin screw extruder (HAAKE MiniCTW, Thermo Fisher Scientific, Germany) and the downstream processing equipment. The blended powders were manually transferred to the preheated barrel and kept for 10 min before extruded from the extruder. The operating temperature changed with formulation as listed in Table 1. The extrudate produced was rod-shaped and about 1 mm in diameter. The rods were cut into implants of 3 mm in length.

2.3. In vitro characterization and evaluation

2.3.1. Differential scanning calorimetry (DSC) analysis

DSC study was carried out to investigate the thermal characteristics of RI. A DSC 822e instrument (Mettler Toledo, Switzerland) was used for the study. Approximately 5 mg of the sample was heated in an open Al-crucible pan at a rate of 10 °C/min within a temperature range of 0–170 °C under a 20 mL/min nitrogen flow.

2.3.2. Scanning electron microscopy (SEM) imaging

The morphologies (surface and cross-section) of the implants retrieved from release medium during release process were imaged by scanning electron microscope (SEM) (JSM-840, JEOL, Japan). The retrieved implants were taken out at preset time. Before visualization, the implants were gently washed with distilled water, blotted with wipes to dry off excess water, and then lyophilized for 48 h. The cross-section of the implants was obtained by first freezing the implants in liquid nitrogen and then breaking off by tweezers. Samples were fixed on metal sample holders and sputter coated with gold using a sputter coater.

2.3.3. Drug loading

RI of each formulation were accurately weighed and dissolved in acetonitrile, and subsequently 0.01 M hydrochloric acid solution was added up to 100 mL. The obtained solution was filtered by a 0.45 μm microporous membrane and then analyzed by HPLC. The mobile phase consisted of acetonitrile and 0.2% H_3PO_4 (40:60, *v*/*v*). The flow rate was maintained at 1 mL/min and UV-detection was performed at 230 nm.

Table 1 Characteristics of the rotigotine-loaded implants formulations.

Formulation	Feed ratios of implant				Melting temperature (°C)	Drug loading (Mean ± SD, n = 10, %)
	Rotigotine (%)	PLGA 7525 5A (%)	PLGA 5050 2A (%)	Additive (%)		
1	20	80	–	–	80	19.1 ± 0.3
2	25	75	–	–	80	24.3 ± 0.5
3	30	70	–	–	80	29.3 ± 0.3
4	35	65	–	–	80	34.1 ± 0.4
5	30	70	–	–	85	28.7 ± 0.6
6	30	70	–	–	90	29.4 ± 0.3
7	30	60	10	–	80	28.4 ± 0.7
8	30	50	20	–	80	29.2 ± 0.4
9	30	40	30	–	80	30.6 ± 0.2
10	30	60	–	Mannitol (10)	85	29.8 ± 0.8
11	30	60	–	Cyclodextrin (10)	85	30.1 ± 0.4
12	30	55	10	Mannitol (5)	85	29.6 ± 0.7
13	30	55	10	Cyclodextrin (5)	85	31.1 ± 0.9

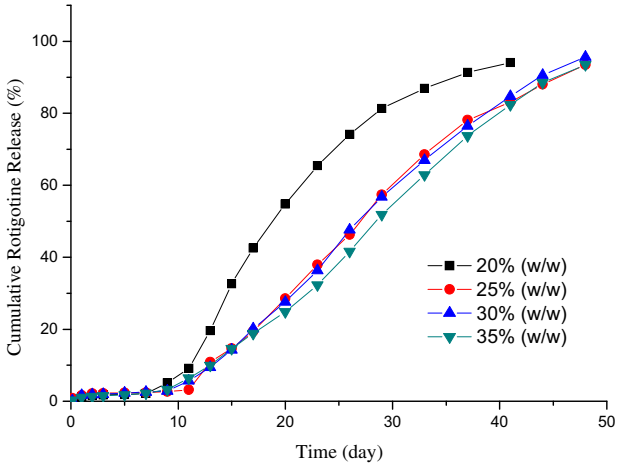

Figure 1 Effect of drug loading (20%, 25%, 30% and 35%) on drug release from RI.

The actual drug loading of implant was then calculated. To evaluate the homogeneity of drug distribution each experimental datum was generated from 10 implants randomly chosen from each batch and expressed as mean value with standard deviation (SD).

2.3.4. In vitro drug release studies

The *in vitro* release study was carried out in triplicate in a shaking water bath. Each implant sample (10–11 mg) was accurately weighed and placed in 25 mL vial containing 10 mL acetate buffer solution (pH = 4.5) with 0.2% SDS and 0.01% Tween-80 under constant oscillation at 50 rpm. At predetermined time points, samples of 8 mL were removed from the vial, and replaced by fresh medium. Samples were analyzed by HPLC method as described above.

2.4. Pharmacokinetics study

Rat (n = 6 per formulation) was injected subcutaneously at the dorsal area with RI at the drug dose of 30 mg/kg/40 d by a special syringe (a syringe for the implant with a needle diameter of 1.1 mm). Blood samples were collected at pre-set intervals before and after drug administration (30 min, 1 h, 3 h, 1 d, 3 d, 5–50 d). The samples obtained were immediately centrifuged at 10,000 rpm and the plasmas were stored at −35 °C before analysis. Rotigotine was analyzed by LC-MS/MS as described previously (Ma et al., 2006). The HPLC system (Agilent 1100 series) consisted of a binary pump and a Zorbax C18 column. The mobile phase was acetonitrile-1 mM ammonium acetate (75:25, v/v) and the flow-rate is 0.30 mL/min. The detection was performed with a TSQ Quantum Access mass spectrometer (Thermo Fisher Scientific Inc., USA) operating in the positive ion mode. Multiple reaction monitoring at unit resolution involved transitions of the protonated forms of rotigotine at m/z 316.2 → 147.1 and diazepam at m/z 285.4 → 193.1. The optimized MS conditions were as follows: curtain gas, gas 1 and gas 2 (all nitrogen) at 15, 50 and 50 psi, respectively; ion spray voltage, 5500 V; source temperature, 500 °C; declustering potentials, 50 V for rotigotine and 160 V for diazepam; the collision gas was set at 3 psi.

3. Results and discussion

3.1. *In vitro* characterization of RI

All implants prepared in this study were smooth and similar in appearance, presenting somewhat tough characteristics. The length of implant was cut into about 3 mm.

Drug contents of RI were analyzed to determine the uniformity of drug distribution and the amount of drug incorporated in each implant. The drug loading of all implants is shown in Table 1. The drug content for all implants was consistent with the theoretical drug-loading, indicating that the implants were uniform with homogenous dispersion and the fabrication process was suitable.

3.2. Effects of drug loading on drug release

The effect of drug loading on drug release from RI was evaluated in this study. Fig. 1 shows the release profiles of RI with different drug loading of 20%, 25%, 30% and 35% (w/w). It was found that drug release from RI with 20% drug loading was faster than the others and there was no significant difference in the release profile among implants with 25%, 30% and 35% drug loading. The drug state existed in the implant was

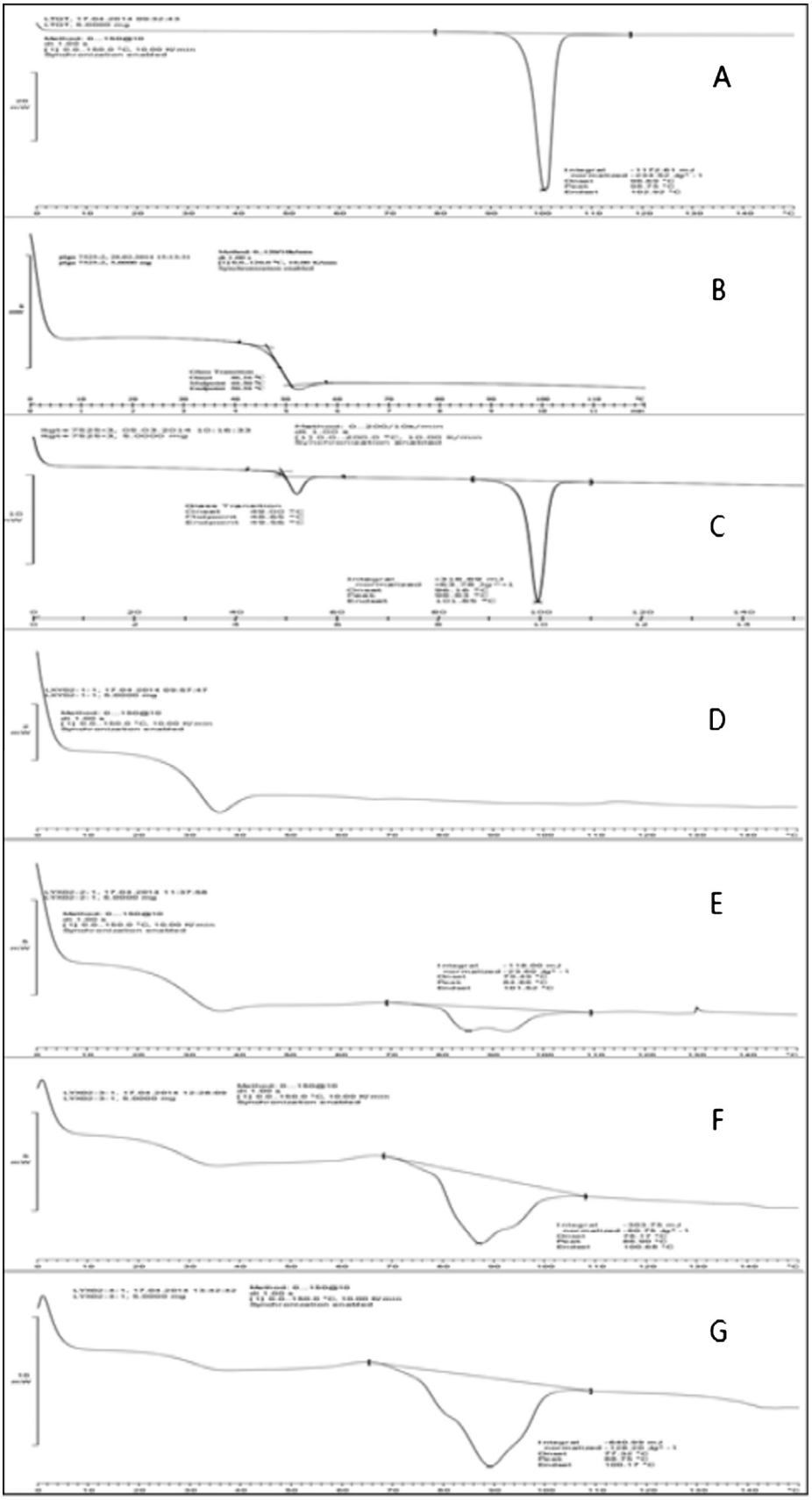

Figure 2 DSC thermograms of rotigotine (A), PLGA 7525 5A (B), the physical mixture of them (C) and RI with 20% (D), 25% (E), 30% (F) and 35% (G) drug loading.

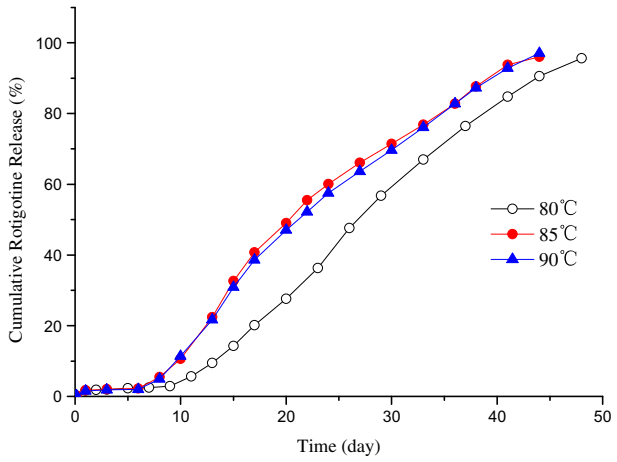

Figure 3 Effect of melting temperature (80 °C, 85 °C and 90 °C) on drug release from RI.

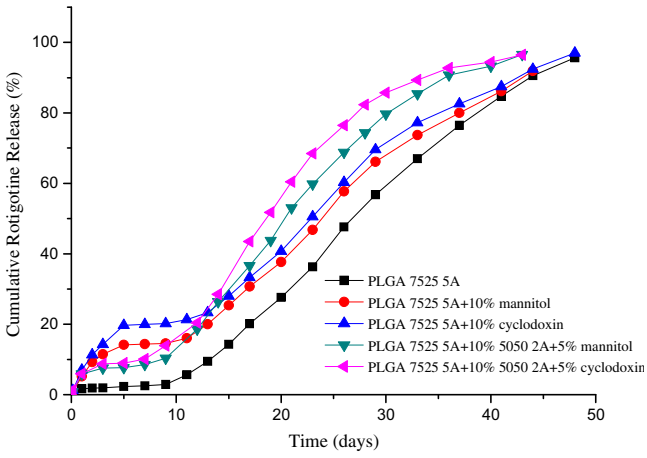

Figure 5 Cumulative release of rotigotine from RI during the *in vitro* release.

investigated by DSC analysis to explore the reason. Fig. 2 displays the DSC thermograms of rotigotine, PLGA, physical mixture of the drug and PLGA and RI with different drug loading. In the DSC thermograms, an endothermic peak of rotigotine was found for RI with 25%, 30% and 35% drug loading, demonstrating that rotigotine existed as a microcrystal state in implants. Furthermore, the peak intensity was proportional to drug loading which indicated that the degree of drug crystallization increased as drug content increased. In contrast, no rotigotine peak was found in DSC thermogram of RI with 20% drug loading, indicating that the amorphous or molecular state of rotigotine dispersed in implants. The difference of drug state in RI with different drug loading may be attributed to the saturated solubility of rotigotine in the PLGA matrix. The results suggested that the drug state existed in the implant is closely related with the drug release.

3.3. Effects of melting temperature on drug release

The influence of melting temperature (80 °C, 85 °C and 90 °C) during preparing process on drug release from RI was also investigated with the same drug loading. As shown in Fig. 3, the drug release from RI prepared by 80 °C melting temperature implants was far slower than 85 °C and 90 °C and there is almost no difference of the release profile between RI prepared by 85 °C and 90 °C melting temperature. DSC analysis (figure not shown) of these implants showed that an endothermic peak of rotigotine was appeared in RI prepared by 80 °C melting temperature which was not observed for RI prepared by 85 °C and 90 °C melting temperature. It was proved that the melting temperature prepared for the implant can affect the drug release by altering the drug physical state existed in the implant. In this study, to achieve slow drug release kinetics, low temperature was used beyond the melting temperature of the materials.

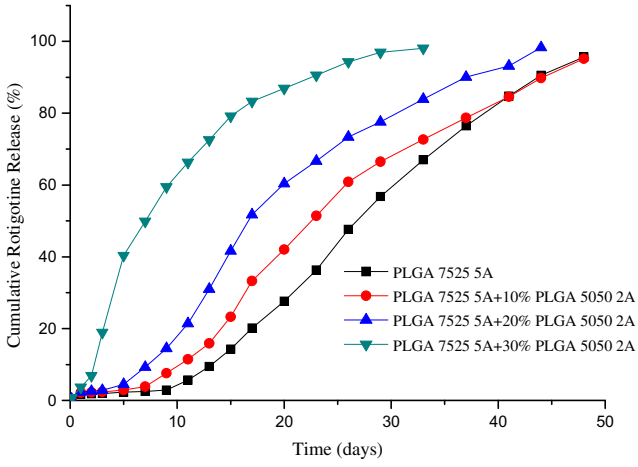

Figure 4 Effect of polymer on drug release from RI.

3.4. Effects of polymer on drug release

The polymer is one of the determined factors to control the rate of drug release from the polymeric matrix. Thus, different implants were prepared by single or blending polymers to optimize the drug release. Fig. 4 shows the *in vitro* drug release profiles of RI prepared by single polymer PLGA 7525 5A and blending polymers of 7525 5A and 5050 2A in different proportions. RI prepared from single polymer of PLGA 7525 5A displayed a 40-day sustained release while with a 10-day lag phase. It was observed that the lag phase was significantly shortened as blending PLGA 5050 2A in the matrix. As the content of 5050 2A increased, the drug release rate increased and the lag phase shortened. This can be attributed to the fast degradation of 5050 2A, which will cause the channels in the matrix. As the content of 5050 2A increased to 30%, the release period was shortened to 30 days.

3.5. Effects of hydrophilic additives on drug release

Addition of certain excipients in the polymeric matrix could influence the drug release significantly by changing the matrix lipophilicity, matrix porosity, microclimate pH and/or polymer drug interactions (Desai et al., 2008; Shyam et al., 2011). Therefore, further investigation was conducted to modify the lag phase by adding hydrophilic substance. The aqueous channels formed by the hydrophilic substance in the

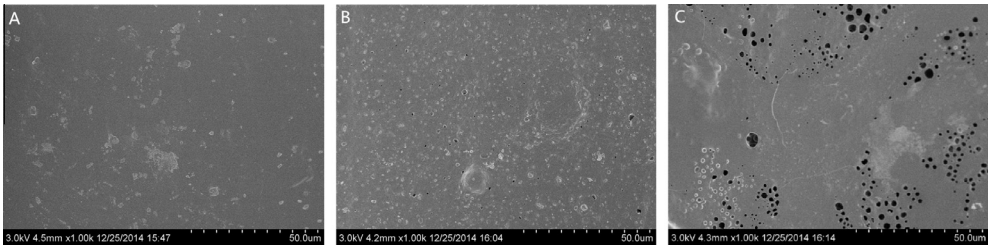

Figure 6 SEM photographs showing surface morphologies of RI. (A) Before drug release; (B) after 3-day drug release; (C) after 12-day drug release. All photographs were obtained at 1000× magnification.

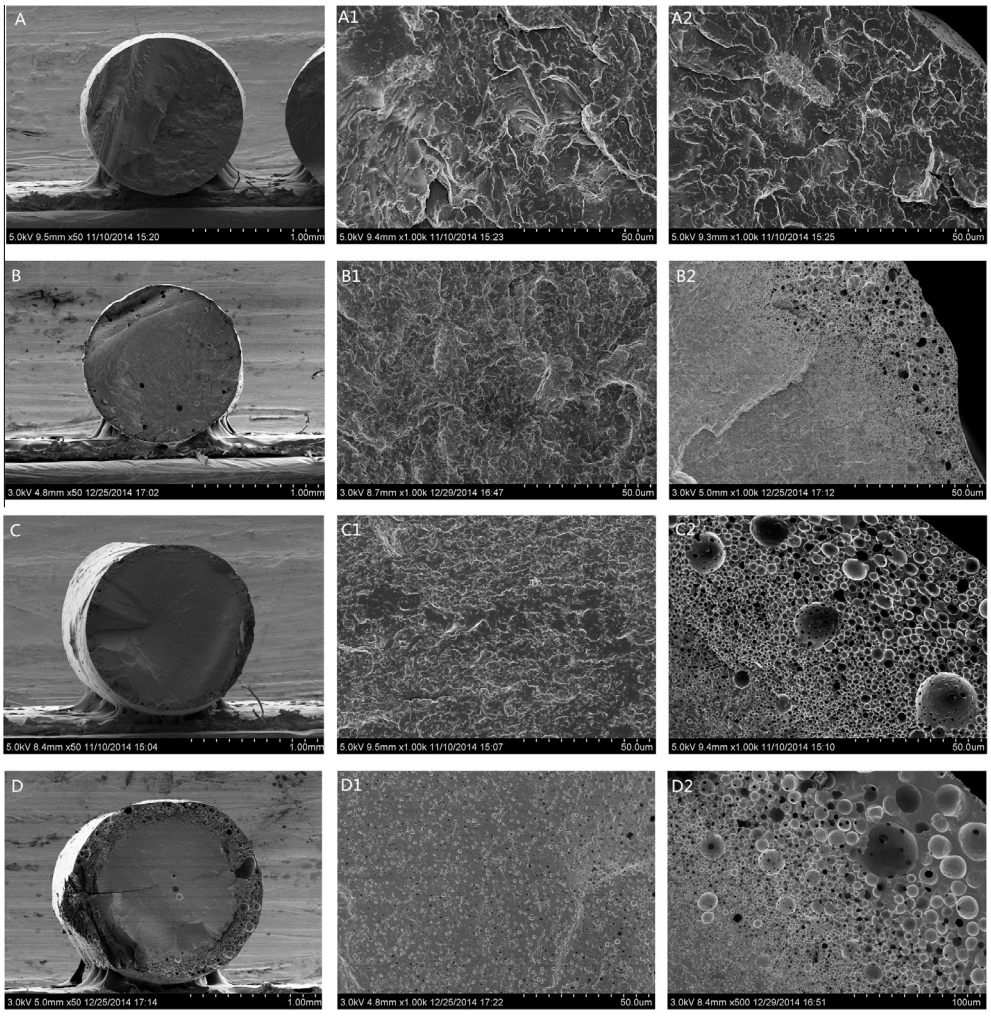

Figure 7 Photographs showing cross-section morphologies of RI of formulation: (A) the whole cross-section (50×), (A1) the interior and (A2) the exterior of the implant before drug release; (B) the whole cross-section (50×), (B1) the interior and (B2) the exterior of the implant after 3-day drug release; (C) the whole cross-section (50×), (C1) the interior and (C2) the exterior of the implant after 9-day drug release; (C) the whole cross-section (50×), (D1) the interior and (D2) the exterior (500×) of the implant after 12-day drug release; All other photographs were obtained at 1000× magnification.

matrix were expected to facilitate the drug diffusion in the initial release to shorten the lag phase (Ma et al., 2006).

Fig. 5 displays the *in vitro* release profiles of RI containing different water-soluble additives (mannitol, cyclodextrin). It can be seen that the drug release from these implants with addition hydrophilic substance follows three-phase release kinetics, initially rapid release, subsequently slow release and then rapid release again. However the lag phase was still existed after initial burst release with the implant prepared with single polymer of 7525 5A. While the implant was prepared with blending polymers of 7525 5A and 5050 2A, the lag phase was significantly shortened in which both the water-soluble additives and 5050 2A played important roles in eliminating the lag phase.

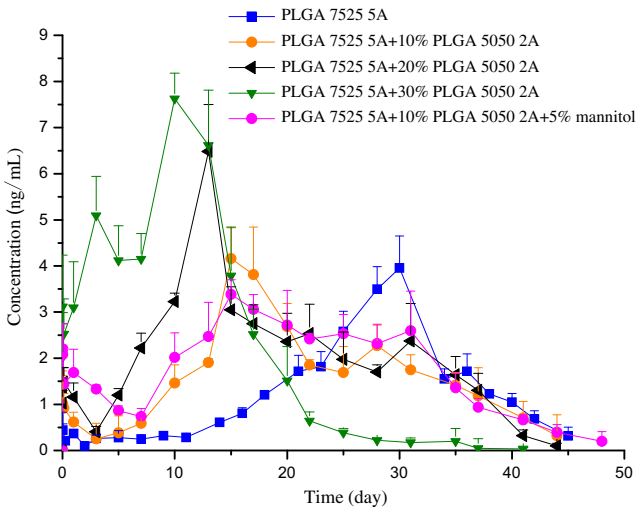

Figure 8 Plasma concentration vs. time curves for rotigotine after administration of RI. Each point represents mean ± SD (n = 6).

3.6. Hydrolytic degradation study

Structural evolution of RI prepared by PLGA 7525 5A during release period was imaged by SEM. The samples before *in vitro* release, after 3, 9 and 12-day release were taken out for SEM study. Samples with longer release period were too vulnerable to remove. Both the surface and the cross-section were dense and devoid of pores before release (Figs. 6A and 7A). After 3-day drug release, a few pores could be observed on the surface of the implant (Fig. 6B). Moreover, it could be seen that a thin layer with osteoporosis structures existed in the surface of the implant, and the internal structure of the implant was almost the same as that before release (Fig. 7B, B1 and B2). After 9-day drug release, it was noteworthy that there existed many pores in the surface of the implants (figure not shown), which was consistent with the exterior of the cross-section morphologies (Fig. 7C2). There were two layers with different structure in the cross-section of the implant and the exterior layer of osteoporosis structure was thicker than before (Fig. 7C). After 12-day drug release, more pores could be seen in the exterior layer of the implant, and the size was larger than before (Fig. 6C). It was obviously that many pores also generated in the interior structure of the implant (Fig. 7D2). These structures were exactly associated with the release profile as shown in Fig. 1 suggesting that the drug release from the implant was controlled by erosion of the matrix and the diffusion of the drug through the layer.

3.7. In vivo drug release

According to the *in vitro* release profiles, five formulations of RI were selected to investigate the in vivo release behaviors on rat (Fig. 8). It was observed that RI prepared with single polymer of 7525 5A displayed a 40-day sustained release while with a lag phase of about 10 days. The addition of PLGA 5050 2A can significantly shorten the lag phase. With the content of 5050 2A increased from 20% to 30%, the lag phase disappeared accompanied by the release period was shortened to 30 days with high initial release. This can be attributed to the fast degradation of PLGA 5050 2A. As the content of 5050 2A increased, the channels formed by the degradation of 5050 2A increased which facilitate the drug diffusion from the internal of the implant and the water molecular penetrates into the PLGA chains promoting the polymer degradation. Formulation 7, which was prepared with 7525 5A/5050 2A/rotigotine = 60:10:30, showed a sustained release period about 40 days. Further, with the addition of mannitol of 5% (Formulation 12), the drug release was more steady which was supposed to achieve continuous dopaminergic stimulation for the treatment of PD with C_{max} of 3.39 ± 0.31 ng/mL on 15th day and the average concentration of 1.94 ± 0.39 ng/mL.

4. Conclusion

In this study, a sustained drug delivery system, rotigotine-loaded implant was successfully developed by hot-melt extrusion method. The optimized formulation of RI can achieve sustained drug release for about 40 days with no lag phase, indicating a good potential CDS treatment of PD. The release of drug from implant was affected by the polymer, drug loading and melting temperature. The structural evolution of implants during release period played an important role in understanding the drug release mechanism. The RI could supply a convenient and practical alternative of CDS treatment for PD.

Acknowledgments

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