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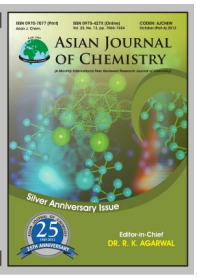

One-Step Synthesis of Polylactide-Dextran Grafted Copolymers and Its Controlled Release on Coenzyme A

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The copolymer of poly(lactide acid)-dextran grafted copolymers (Dex-g-PLA) was synthesized by one-step melting reaction method under microwave irradiation. Compared with two-step reaction method, the copolymerization rate was accelerated under microwave irradiation. The conversion of monomer reached 46 % and graft rate reached 142 % after 15 min under microwave irradiation. The influence of different parameters affecting the synthetic process, namely monomer-to-initiator ratio, reaction time, different concentration of catalyst, reaction time and temperature of solvent were investigated. The final products characterized by infrared spectroscopy, nuclear magnetic resonance and powder X-ray diffraction are proved that it indeed is Dex-g-PLA copolymers. Then Dex-g-PLA microspheres loaded with coenzyme A were prepared by double emulsion method. The drug controlled release experiment *in vitro* demonstrates that after 21 days, the release rate of Dex-g-PLA coenzyme A microspheres reaches 83 % and after 30 days, the release rate reaches 87 % with the saturated release rate.

Key Words: Controlled release, Graft copolymer, Microwave irradiation, One-step synthesis, Polylactide.

INTRODUCTION

Poly(lactide acid) (PLA) are considered as the most versatile materials among biodegradable polymers because of its inherent biodegradability, biocompatibility, high mechanical strength and the easy availability. Currently, PLA polymers are the most widely used synthetic biodegradable polymer for biomedical applications and pharmaceutical industries[1]. It has been approved by US FDA since 1970s and has been widely utilized in sutures, implant material, wound closure[2,3], bone fixation devices and a microcapsule vehicle for controlled drug delivery[4-7]. However, when it was considered as practical requirements of tissue engineering and drug delivery systems, many obvious disadvantages are usually insufficient to satisfy the requirement of most practical applications, such as the poor hydrophilicity of the PLA greatly affects cell adhesion onto the surface and penetration into the scaffolds. The degradation rate of the PLA also may not meet the different requirements of various tissue engineering scaffolds. Therefore, in order to overcome the shortcomings of the PLA and make it more promising in pharmaceutical applications, the modification of PLA has attracted more and more interesting[8-12]. Polysaccharides such as dextran (Dex), with many hydroxyl groups, are important natural biodegradable hydrophilic polymers with enzymatic degradation behaviours and good biocompatibilities. The modification of PLA by introducing hydrophilic and biocompatible dextran can enhance its hydrophilicity because of the introduction of hydrophilic glucose units[13]. The biodegradable polylactide-dextran grafted copolymers (Dex-g-PLA) can combine a dextran backbone together with polylactide grafts. A well-controlled synthesis procedure can allow the preparation of various grafted copolymers[5,6], which are bound to influence either the number of polylactide side chains attached to the dextran or the length of the PLA grafts. Thus, by modulating the ratio of PLA/ dextran, the graft copolymers may have both highly soluble in water and a satisfactory rate of enzymatic degradation, which may be beneficial for excellent biocompatibilities, biodegradabilities and cell affinities[14-16].

The biodegradable polylactide-grafted dextran copolymer (Dex-g-PLA) can be synthesized by some specific methods. The currently reported controlled synthesis of amphiphilic Dex-g-PLA is by using a three-step strategy or by bulk polymerization with trimethylsilyl (TMS)-protected dextran as a macro initiator. All of those methods may cost longer time, high cost and low production efficiency[17]. For example, the

three-step procedure included partial silylation of the dextran hydroxyl groups, ring-opening polymerization of D,L-lactide initiated from remaining hydroxyl groups, silylether deprotection under very mild conditions. Therefore, it is important to find some new synthetic methods to maintain the PLA grafts readily undergoing hydrolysis under convenient conditions.

Nowadays, as an alternative to conventional heating, the microwave irradiation (MI) recently has become a popular in organic chemistry[18-21]. The microwave irradiation heating can ensure the polymerization process to perform very well. The polymerization under microwave irradiation has the advantages of higher reaction rate and greater yield of polymer within a shorter reaction time; namely, it can evidently enhance the reactivity of reaction with the fewer side reactions. To our best of knowledge, there have been no reports on copolymer of Dex-g-PLA under microwave irradiation.

In this study, Dex-g-PLA copolymer was performed by one-step melting synthetic pathway under microwave irradiation. The optimized process is finally proposed as feed ratio (LLA/Dex mass ratio) 4:1, catalyst 3 wt %, reaction temperature 135 ºC and reaction time 15 min with the help of microwave irradiation. In additional, coenzyme A, as a model drug, was then encapsulated into the prepared Dex-g-PLA polymer as microspheres by multiple emulsion method[22] and their *in vitro* drug delivery release behaviour under the simulating *in vivo* environment conditions was also showed good controlled release characteristics of the polymer.

EXPERIMENTAL

L-Lactide (LLA, analytial reagent) and Dextran-20 were purchased from Tianjin Bodi Chemical Industry Limited Company. L-Lactide was recrystallized twice with dry toluene and dried under vacuum before use. Dextran-20 was dried and distilled. Stannous octoate ($Sn(Oct)_2$, analytical reagent) and trimethylchlorosilane (Chemical pure) were purchased from Sinopharm Chemical Reagent Co., Ltd, China. After diluted in dry toluene, $Sn(Oct)_2$ solution was stored in glass ampoules under nitrogen. Dimethyl sulfoxide (analytical reagent) was purchased from Xilong Chemical CO., Ltd., China. Poly(vinyl alcohol) (PVA) was from Shanghai Crystal Pure Reagent Limited Company. Potassium bromide (spectrum pure) was purchased from Shanghai Pharmaceutical Company. The coenzyme A were purchased from Sigma-Aldrich (USA) and used without any further purification. All other reagents were used without further purification.

Dex-g-PLA copolymer synthesis: The typical preparation procedure of Dex-g-PLA copolymer described as follows: a dry round-bottom flask equipped with a three-way stopcock was stowed with dextran-20 and L-lactide according to different ratio of Dex to LLA (1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8). The flask was sealed and degassed by means of three freeze-pump thaw cycles at 45 ºC for 50 min, filled with nitrogen and heated. After stirred evenly (500 rpm) under the microwave energy exposure, the appropriate amount of initiator, $Sn(Oct)_2$, was added. The frequency of microwave is 2450 MHz with the power of 450 W (Xywb9-MAS-3, Westernization Instrument Limited Company of Science and Technology, China). After reaction, the flask was cooled by water. A small amount of DMSO was introduced to dissolve the residue. The L-lactide monomers and homopolymer can be obtained by ethanol precipitation. The precipitate mixture was then extracted by methanol solvent in Soxhlet extractor to remove the lower weight polylactide. After vacuum freeze-drying (FCM50D Freeze Drier, Steris, USA), the pure Dex-g-PLA copolymer can be obtained. The detailed synthesis process and schematic diagram are shown in Fig. 1. The different synthetic conditions

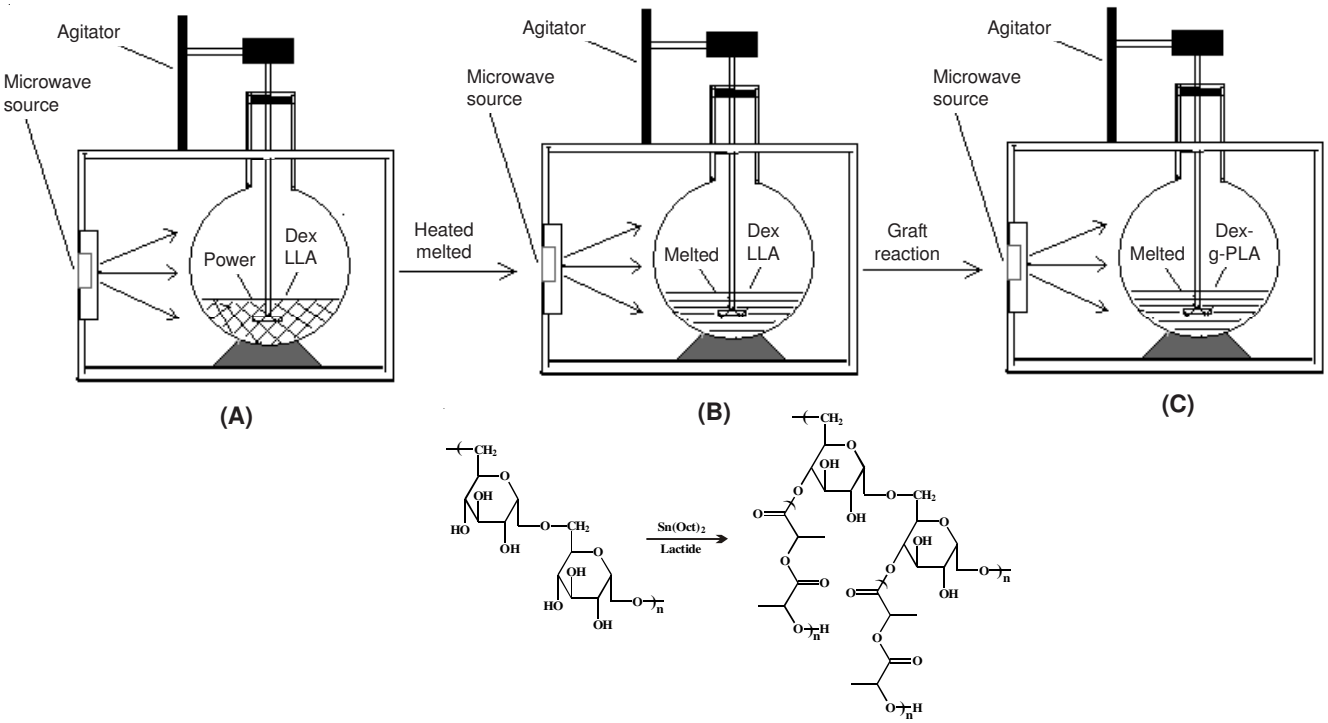

Fig. 1. Process and schematic diagram of equipment for Dex-g-PLA polymer synthesis under the microwave irradiation by one melting method. The microwave exposure can be useful for the polymer heating and graft reaction

including the feed ratio of Dex to L-lactide, the catalyst amount, the reaction time and reaction temperature were further investigated.

Characterization of Dex-g-PLA: The structure of obtained Dex-g-PLA copolymer was verified by Fourier transform infrared (FTIR) spectra of a Vector-22FT-IR FTIR spectrophotometer (Nicolet5700, The Thermo Company, USA) with the scanning range of 4000-400 cm^{-1}. The X-ray images were obtained by X-ray diffractometer (D/Max-rB, Rigaku Company, Japan). ^1H NMR (400 MHz) spectra were recorded on a Bruker Avance spectrometer with DMSO as solvent at room temperature.

Preparation of coenzyme A loaded Dex-g-PLA microsphere: The synthesis of Dex-g-PLA microspheres loaded with coenzyme A has been briefly reported as follows. Firstly, the Dex-g-PLA (15 g) was dispersed in dichloromethane as the oil phase. Coenzyme A (0.6 g) was fully dissolved into the gelatin solution (20 %) as the water phase and kept under the constant temperature of 40 ºC. Then the oil phase was added into the water phase and mixed evenly. After ultrasonication and stirring for 5 min, the first emulsion mixture was obtained. When the first emulsion mixture was cooled down to 12-15 ºC, the poly(vinyl alcohol) solution (4 %) was immediately added to the first emulsion and stirring for 10 min with the rotation of 1,500 rpm, the multiple emulsion solution was achieved. Subsequently, in order to remove the organic solvent completely, the multiple emulsion solution was added to the poly(vinyl alcohol) solution (0.1 %) at 30 ºC. After isolation by centrifugation under the rotation of 3,000 rpm for 10 min, the Dex-g-PLA coenzyme A microspheres were dried by vacuum freeze-drying (FCM50D Freeze Drier, Steris, USA). When using, the product was redispersed into water at room temperature. The size and morphology of the products were characterized by scanning electron microscope (SEM, JEOL, JSM-6700F, Japan) and laser particle size analyzer (Nano-ZS90, Hitachi, Japan), respectively.

***In vitro* release of coenzyme A from the Dex-g-PLA microshperes:** To measure the concentration of drug release, 0.02 g microsphere sample was put into the dialysis bag and then put into the constant temperature oscillator (HZQ-F160A, China) at the 100 r min^{-1} rotational speed under the temperature of 37 ± 0.5 ºC. The total detection time was 30 days and 5 mL solution from the constant temperature oscillator was sampled every 3 day. The measurement of coenzyme A concentration was according to the reported methods[18]. After calculating the cumulative release rate, the controlled release rate of coenzyme A from the Dex-g-PLA can be obtained.

RESULTS AND DISCUSSION

The experiment investigated the feed ratio of dextran to poly(lactide acid) (weight ratio), catalyst amount, reaction time and temperature. The optimization parameters were studied based on the graft rate and conversion efficiency. The grafting ratio and conversion efficiency were calculated by the following equations:

$$\text{Graft rate} = \frac{(W_1 - W_2)}{W_2} \quad (1)$$

$$\text{Conversion efficiency} = \frac{(W_1 - W_2)}{W_3} \quad (2)$$

where W_1 is the weight of obtained Dex-g-PLA, W_2 is the weight of dextran before grafting and W_3 is the weight of lactide before grafting.

Influence of the feed ratio of dextran to L-lactide: Under the same temperature, time and catalyst amount, the different feed ratio of dextran to L-lactide (1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8) was explored. The results are shown in Fig. 2. With the increase of feed ratio, the graft rate and conversion rate are increased before the ratio of 1:6. When the ratio is between 1:4 and 1:6, the increase amplitude becomes slow. To the ratio of 1:6, the graft rate is the maximum. Then over the ratio of 1:6, the efficiency begins to decrease. At the same time, the conversion efficiency maintains on the same level. Besides, different feed ratio may lead to different morphology. When the ratio is 1:2, 1:3, 1:4, the product is the power. When the ratio is 1:5, 1:6, the product is the sticky solid. When the ratio reaches 1:7 and 1:8, the viscosity of the product is the very big. If such product was kept under the room temperature for a longer time, it would be melted. Therefore, by comprehensive consideration, the ratio of 1:4 is the best one.

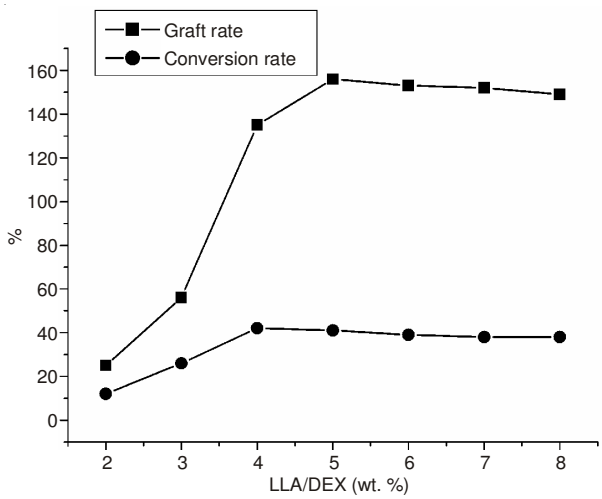

Fig. 2. Feed ratio of LLA to dextran. With the increase of the ratio between them, the graft rate and conversion rate can be significantly changed. The ratio of 1:4 for LLA to dextran would the best for comprehensive consideration

Influence of catalyst amount: Under the same feed ratio, temperature, time, the different catalyst amount was also studied (1, 2, 3, 4, 5 and 6 wt %) (Fig. 3). With the increase of the catalyst amount, the graft and conversion rate are increased. When it reaches 5 wt %, the efficiency has a little decrease. The reason may be that the $Sn(Oct)_2$ can both be catalyst and depolymerizing agent. By changing the catalyst amount, the molecular weight of the PLA can be obtained. A large amount of $Sn(Oct)_2$ would induce the lower molecular weight of PLA. The results show that when the amount is 3,4 and 5 wt %, there is similar efficiency. In order to lower the production cost, the appropriate catalyst amount was chosen to be 3 wt %.

Influence of reaction time and temperature: Based on the single factor experimental results, the graft rate and conversion efficiency of the Dex-g-PLA will change with the reaction time and temperature. In order to find out the appropriate reaction time and temperature, two factors and three levels of optimization method had been applied (factorial design), which

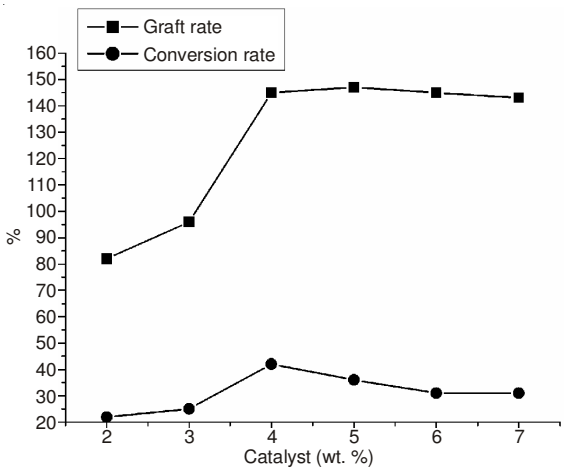

Fig. 3. Study of catalyst amount. In order to lower the production cost, 3 wt % was chosen as the appropriate catalyst amount

include the time (10, 15 and 20 min) and temperature (130, 135 and 140 ºC). Table-1 shows the factors and levels. According to the full factor design (Table-2), there should be nine experiments to investigate the graft rate and conversion efficiency.

TABLE-1
FACTORS AND LEVELS DESIGN FOR THE SYNTHESIS OF PLA. TWO FACTORS WERE CHOSEN WITH THREE LEVELS EACH

Factors	Level		
	Low	Media	High
Temperature (ºC)	130(A)	135(B)	140(C)
Time (min)	10(I)	15(II)	20(III)

TABLE-2
FACTORS AND LEVELS DESIGN USING ORTHOGONAL TABLE

Experimental numbers	Factors		Results	
	Temperature (ºC)	Time (h)	Graft rate (%)	Conversion rate (%)
1	A	I	112	36
2	A	II	119	42
3	A	III	118	41
4	B	I	112	33
5	B	II	142	46
6	B	III	126	44
7	C	I	89	23
8	C	II	98	26
9	C	III	99	28

From the results of Table-2, F_1-0.05(2, 4) = 6.94. By variance analysis[23], in order to obtain high graft rate, the F = 15 > 6.94. In order to obtain better conversion rate, the F = 8 > 6.94. The results indicate that there is significant differences among the different reaction temperature ($p < 0.05$). In the experiments, it is found that when the reaction temperature increases up to above 130 ºC, the mixture of dextran and the L-lactide becomes melting and begins to react. When the mixture totally melts, the graft rate and conversion rate would increase with the reaction temperature. After the mixture is melted, the reaction turns to exothermic reaction, which is not beneficial for the reaction. During the reaction progress, the graft rate and the conversion rate begin to increase and then decrease with the increase of the reaction temperature. Therefore, the reaction temperature of 135 ºC is chosen as the appropriate condition.

When considering the influence of reaction time, in order to obtain high graft rate, the F = 3 < 6.94. In order to obtain better conversion rate, the F = 2 < 6.94. The result indicates that there is no significant differences among the different reaction time ($p > 0.05$), which shows that the longer reaction time results in the lower efficacy. Therefore, the appropriate reaction time is 15 min. Under the temperature of 135 ºC with the help of microwave irradiation, the graft rate can reach 142 % and conversion efficiency reaches 46 %.

FTIR spectra of Dex-g-PLA: Fig. 4 is the FTIR spectra of obtained product. By comparing spectra of the dextran and Dex-g-PLA, the additional strong absorption peaks appeared at 1760 cm^{-1}, which was the characteristic absorption of ester bond carboxyl group. The FTIR result indicates the success of the one-step melting synthesis for Dex-g-PLA.

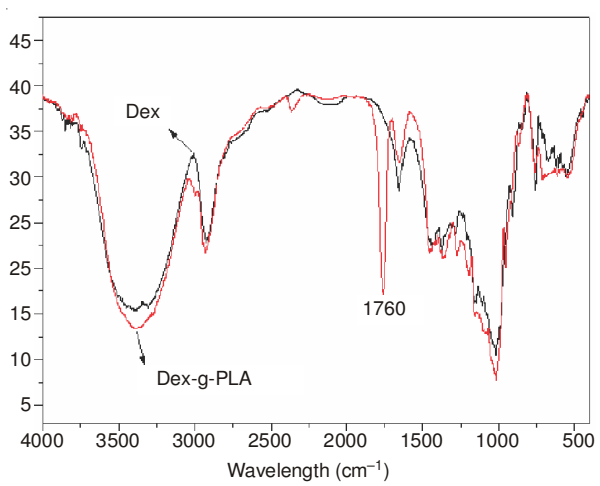

Fig. 4. FTIR spectra of dextran and Dex-g-PLA. The additional strong absorption peak at 1760 cm^{-1} show that one-step melting synthesis method can be successfully used for to produce Dex-g-PLA copolymer

^{1}H NMR spectra of Dex-g-PLA: A typical ^{1}H NMR spectrum (Fig. 5), confirms the formation of the Dex-g-PLA copolymers. As shown in Fig. 5, the peak at 5.17 ppm (s, 1H) originates from the methyl side-group on the PLA. The peak at 1.46 ppm is attributed to the hydrogen atoms on methylene groups. The peak at 4.3 ppm is the end hydrogen of dextran. The weak broad peak ranging from 3.5-3.9 ppm is the methylene and methane on the dextran. The peak of 2.0 ppm is the solvent peak. The ^{1}H NMR spectra proves the existence of Dex-g-PLA.

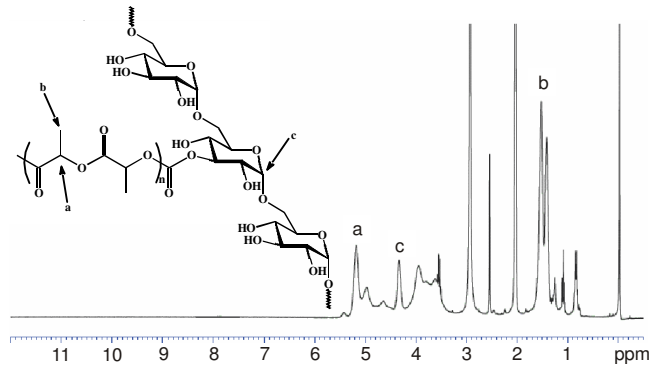

Fig. 5. ^{1}H NMR spectra of Dex-g-PLA, which proves the existence of the Dex-g-PLA copolymer

X-Ray diffraction analysis: The powder XRD measurements were performed on an X-ray diffractometer with graphite monochromatized CuK_α radiation (k = 0.15406 nm). The acceleration voltage was 40 kV with a 100 mA current flux. A scan rate of 1º was used to record the patterns in the 2 h range of 5-60º.

The powder XRD pattern of the Dex-g-PLA is shown in Fig. 6. One can see that the best results (the lowest intensity of Dex-g-PLA peaks) have been obtained. The peaks of dextran are 15.3, 17.4, 18.7, 20.1 and 28.9º and the peaks of Dex-g-PLA are 31.7, 45.5 and 56.5º. The well-defined, sharp diffraction lines suggest the well crystallized Dex-g-PLA.

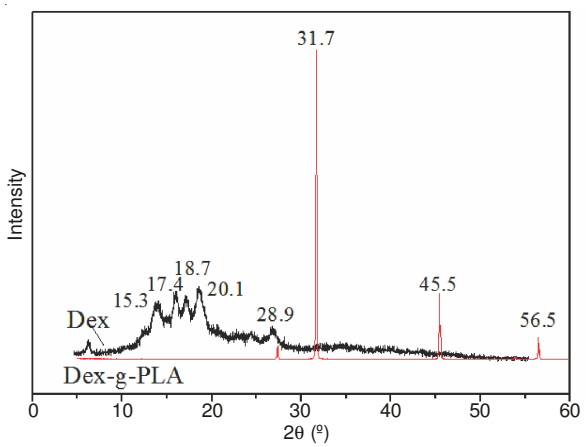

Fig. 6. Powder XRD patterns of dried Dex-g-PLA. The peaks of Dex-g-PLA were at 31.7, 45.5 and 56.5º

Controlled release of Dex-g-PLA coenzyme A microspheres: The SEM images of the microspheres are shown in Fig. 7(a-c), which shows different magnification with 200, 2,000 and 20,000 respectively. The morphology of the microspheres is spherical and there are many little pores on the Dex-g-PLA encapsulated surface, which plays an important role in the controlling of the drug from the microshperes. And the size distribution results are shown in Fig. 7g. The mean diameter of the coenzyme A loaded microspheres is *ca.* 38 μm with the volume size distribution ranging from 20-60 μm.

The controlled release rate of coenzyme A from the microspheres is shown in Fig. 7h. Because the controlled release curve is similar with the Hyperbl function, a new power law model, $y = Ax^{n1}/(B + Cx^{n2})$, can be considered as the coenzyme A release function[24]. After fitted by the statistical Origin 8.0 software, the drug release kinetics can be described as $Rc = 100t^{0.42}/(0.16 + 7.74t^{1.24})$, the correlation coefficient R^2 is 0.9997. The results indicate that after 3 days, the significant release of the coenzyme A begins and the cumulative release rate reaches 29 %, then the release rate becomes slow, until 9 days, the release rate reaches 58 and 74 % after 15 days. On the 21 days, the release rate reaches 83 % and after 30 days, the release rate reaches 87 % with the saturated release rate. The cumulative release rates results demonstrate that under the simulating *in vivo* environment, the coenzyme A loaded with Dex-g-PLA microspheres remain the sustained release effect. The SEM morphology of the microsphere after 3 days, 15 and 21 days also prove the results (Fig. 7d-7f). With the process of the drug release, the drug can be released

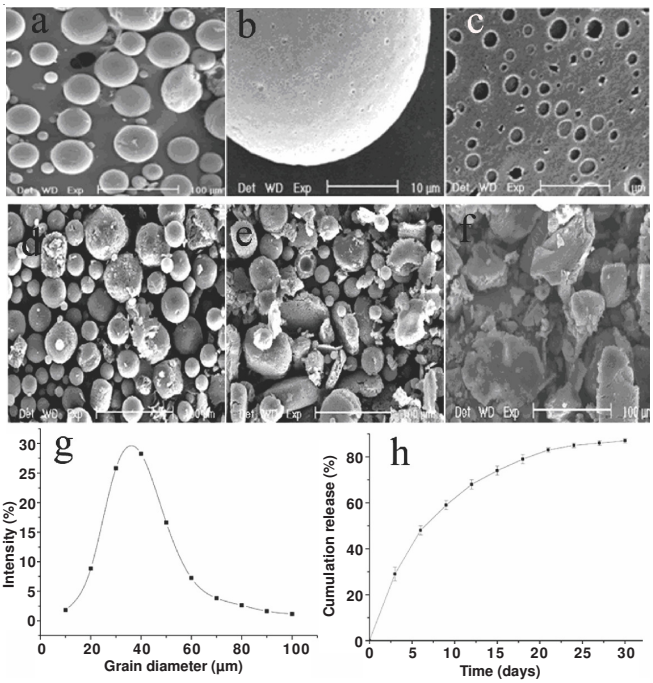

Fig. 7. SEM images of the coenzyme A loaded microspheres. (a-c) are the images of microspheres samples before release under the magnification of 200, 2,000 and 20,000, respectively. (d-f) The SEM morphology of the microsphere after 3 days, 15 days and 21 days release in the solution. The coenzyme A can be controlled release from the fractured microspheres. (g) The DLS results of the coenzyme A microspheres. (h) The cumulative release rate of coenzyme A from the Dex-g-PLA micrspheres. (n = 3)

from the microspheres because the PLA shells of the microspheres are disrupted during the hydrolysis.

Polylactide-dextran grafted copolymers synthesized by one-step mainly attribute to the help of microwave energy. The synthesis of Dex-g-PLA copolymer mainly relies on the ring opening polymerization process, which is coordination-insertion polymerization mechanism[25,26]. Under the microwave irradiation and heating,the dextran with the abundant hydroxy bonds can fully melted into the L-lactide monomers, which is beneficial for producing the Dex-g-PLA copolymer. Therefore, the microwave irradiation plays an important role in the one-step synthesis process. The reason may be that the polar medium can generate molecular agitation because of the high-speed rotating resulting from the microwave irradiation. Besides the microwave irradiation can heat the medium more evenly, which prompts some organic compounds adsorb around on the samples. The emerged local "hot spot" makes the reaction become more quickly and more successfully. By using this reaction setup, the optimal dimensions and position of the reaction bottom inside the cavity were adjusted to enable the homogeneous irradiation of the whole sample. Therefore, because of advantageous features such as shorter reaction times, greater yields, limited generation of by-products and relatively easy and straightforward scaleup, microwave-assisted synthesis may be a very appealing tool in Dex-g-PLA copolymer synthesis.

Conclusion

Poly(lactide acid)-grafted dextran copolymers (Dex-g-PLA) were successfully synthesized by one-step melting

method with the help of microwave irradiation. The objective compound has been characterized by FTIR and NMR. The X-ray diffraction has also proved the crystalline form of Dex-g-PLA. By single-factor experiments, the optimum synthetic conditions are achieved as follows: the feed ratio of dextran to L-lactide is 1:4, the catalyst amount is 3 wt % (weight ratio), reaction time is 15 min and temperature is 135 ºC. The Dex-g-PLA can have graft rate of 142 % and conversion rate of 46 %. Under these conditions, the prepared Dex-g-PLA can have good physicochemical properties within the shorter reaction time because of the existence of microwave energy. After the coenzyme A was encapsulated in the Dex-g-PLA by multiple emulsion methods, the controlled release rate can be obtained. The prepared PLA-grafted dextran copolymers may be good drug delivery carriers. Therefore, the proposed one-step melting method under the microwave irradiation makes the synthetic process become more effective, convenient and time saving.

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REFERENCES

1. P.B. Maurus and C.C. Kaeding, *Oper. Tech. Sports Med.*, **12**, 158 (2004).
2. T.G. Park, *Biomaterials*, **16**, 1123 (1995).
3. M. Fini, S. Giannini, R. Giordano, G. Giavaresi, M. Grimaldi, N.N. Aldini, L. Orienti and M. Rocca, *Int. J. Artif. Organs*, **18**, 772 (1995).
4. S. Zhou, X. Liao, X. Li, X. Deng and H. Li, *J. Control. Rel.*, **86**, 195 (2003).
5. J. Slager and A.J. Domb, *Biomaterials*, **23**, 4389 (2002).
6. L. Valérie, V. Karine, J.J. Peron, A. Borgne, M. Walls and P. Guerin, *Polym. Degrad. Stab.*, **76**, 411 (2002).
7. C. Perez, A. Sanchez, D. Putnam, D. Ting, R. Langer and M.J. Alonso, *J. Control. Rel.*, **75**, 211 (2001).
8. B. Baras, M.A. Benoit and J. Gillard, *Int. J. Pharm.*, **200**, 133 (2000).
9. T. Chung, Y. Huang and Y. Liu, *Int. J. Pharm.*, **212**, 161 (2001).
10. F. Tasaka, H. Miyazaki, Y. Ohya and T. Ouchi, *Macromolecules*, **32**, 6386 (1999).
11. T. Tarvainen, T. Karjalainen, M. Malin, S. Pohjolainen, J. Tuominen, J. Seppälä and K. Järvinen, *J. Control. Rel.*, **81**, 251 (2002).
12. M.K. Das, K. Husain and Y. Pathak, *Asian J. Chem.*, **25**, S297 (2013).
13. D.F. Karin, J.H. Thijs and J. Wim, *J. Control. Rel.*, **111**, 117 (2006).
14. J. Raynaud, B. Choquenet, E. Marie, E. Dellacherie, C. Nouvel, J.L. Six and A. Durand, *Biomacromolecules*, **9**, 1014 (2008).
15. K. Nagahama, Y. Mori, Y. Ohya and T. Ouchi, *Biomacromolecules*, **8**, 2135 (2007).
16. D.E. Noga, T.A. Petrie, A. Kumar, M. Weck, A.J. García and D.M. Collard, *Biomacromolecules*, **9**, 2056 (2008).
17. J.C. Middleton and A.J. Tipton, *Biomaterials*, **21**, 2335 (2000).
18. G. Wang and H. Wu, *Polym. Bull.*, **67**, 1809 (2011).
19. Z. Li, F. Shi, Y. Ding, T. Zhang and C. Yan, *Langmuir*, **27**, 14589 (2011).
20. G.A. Gotelli, P. Bonelli, G.A. Abraham and A Sosnik, *J. Appl. Polym. Sci.*, **121**, 1321 (2011).
21. F. Rafiemanzelat and E. Abdollahi, *J. Appl. Polym. Sci.*, **123**, 1015 (2012).
22. J. Pan, M. Zhao, Y. Liu, B. Wang, L. Mi and L. Yang, *J. Biomed. Mater. Res. A*, **89**, 160 (2009).
23. Q. Ping and Y. Hu, J. Zhou China Medical Science and Technology Press, Beijing, China (1998).
24. R. Yao, Doctoral Dissertation, China (2009).
25. H.R. Kricheldorf, I. Kreiser-Saunders and A. Stricker, *Macromolecules*, **33**, 702 (2000).
26. K. Kempe, C.R. Becer and U.S. Schuber, *Macromolecules*, **44**, 5825 (2011).