Polym. Bull. (2012) 68:27–36
DOI 10.1007/s00289-011-0516-x

ORIGINAL PAPER

Synthesis and characterization of star-shaped poly (lactide-*co*-glycolide) and its drug-loaded microspheres

Chunping Ouyang · Qian Liu · Shunxin Zhao · Guilei Ma · Zhengpu Zhang · Cunxian Song

Received: 13 December 2010 / Revised: 25 April 2011 / Accepted: 22 May 2011 /
Published online: 10 June 2011

Abstract The star-shaped poly (lactide-*co*-glycolide) (PLGA) was synthesized via the ring-opening polymerization of D,L-lactide and glycolide, with pentaerythritol as a multifunctional initiator and stannous 2-ethyl hexanoate as a catalyst. The structures of these polymers were characterized by ^{13}C-NMR spectroscopy, while the molecular weight and polydispersity index (PDI) were determined by gel permeation chromatography (GPC). The glass transition temperature (T_g) of copolymer was determined by differential scanning calorimetry (DSC). Bovine serum albumin (BSA) loaded microspheres were fabricated using star-shaped PLGA by a W/O/W double emulsion solvent evaporation method. The results of characterization demonstrated that the particle size of the PLGA microspheres were about 80–150 μm, the maximum loading capacity and encapsulation efficiency of BSA-loaded microspheres were 67.51 μg/(mg microspheres) and 78.39%, respectively, which were better than linear PLGA. The in vitro release profiles of BSA in phosphate buffer saline (PBS) lasted for 37 h. Drug release profiles can be affected by polymer molecular weight and the ratio of polymer to drug. The maximum release percentage was 80%.

Keywords BSA · Drug-loaded microspheres · Encapsulation efficiency · Loading capacity · Star-shaped PLGA

C. Ouyang · Q. Liu · S. Zhao · Z. Zhang (✉)
Key Laboratory of Functional Polymer Materials, Ministry of Education, and Institute of Polymer Chemistry, Nankai University, Tianjin 300071, China
e-mail: zhangzp@nankai.edu.cn

G. Ma · C. Song (✉)
Institute of Biomedical Engineering, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China
e-mail: scxian@tom.com

Introduction

Controlled drug delivery systems have attracted more and more attention during the past few decades [1–6]. Poly (lactide-*co*-glycolide) (PLGA), which is known to be non-stimulating, non-toxic, biocompatible and biodegradable biomaterial, is now widely used for drug release. PLGA as a carrier material has been used to package low-molecular-weight drugs, peptide, proteins, biological macromolecules, drugs, and vaccines [7–11]. However, the loading capacity (LC) and encapsulation efficiency (EE) of microspheres fabricated with linear PLGA were not satisfaction [12].

Now, PLGA prepared was mainly the linear-based copolymer, rather than in a single linear polymer, the pre-designed shape of the polymer has attracted more and more interest of researchers, such as branched, star, dendritic, and so on. And it found that these branched polymer structure would bring many physical, chemical, biological and other performance improvements. There were many reports related to the synthesis of star-shaped PLGA recently [13, 14]. Dong et al. [15–17] have reported the synthesis and characterization of three-arm and four-arm, star-shaped PLGA, but they did not conduct their application in drug release. A water-in-oil-in-water (W/O/W) double emulsion solvent evaporation method is one of the most popular methods used to encapsulate water-soluble drugs, particularly protein and peptide drugs [18–23].

In this article, the star-shaped poly (lactide-*co*-glycolide) (PLGA) was synthesized via the ring-opening polymerization of D,L-lactide and glycolide, with pentaerythritol (PTOL) as a multifunctional initiator and stannous 2-ethyl hexanoate as a catalyst. Bovine serum albumin (BSA) as a drug model was applied to the preparation of drug-loaded microspheres. For comparison, the linear PLGA was used to analyze the influencing factors of drug containing and drug release.

Experimental

Materials

Glycolide (m.p. 83.5–84.5 °C) and D,L-lactide (m.p. 126.5–127.5 °C) were purchased from Beijing Yuan-Sheng Rong Technology Co., LTD. (China). BSA (98% pure by gel electrophoresis, 68 kDa) was obtained from United Stars (China). PTOL (AR) was supplied by Tianjin Fuchen Chemistry Reagent factory (China). Dichloromethane was distilled after the pretreated with anhydrous $MgSO_4$. $Sn(Oct)_2$ (Sigma 95%) was diluted to desired concentration (1 g/mL) with dichloromethane. l-PLGA ($M_w \sim 10{,}000$ Da) was made by ourselves [24]. All other chemicals were of analytical grade and used without further purification.

Instruments

The measurements of the ^{13}C-NMR spectra were performed on a VARIAN UNITY-plus 400 MHz NMR spectrometer (Varian, USA) with $CDCl_3$ as the solvent. The

weight-average molecular weight (M_w) and polydispersity index (PDI) of the copolymers were determined with a Waters 510 gel permeation chromatography with HPLC grade THF as the solvent and polystyrene as the standard. Specimen concentrations were 2.5–5 mg/mL, and the flow rate was 1 mL/min at 30 °C. Differential scanning calorimetry (DSC) was performed on the NETZSCH differential scanning calorimetry with 2–3 mg sample. DSC scans were carried out over the temperature range from 0 to +80 °C at a heating rate of 5 °C/min, kept down the data. SEM micrographs were obtained with FEI QUANTA 200 scanning electron microscope. The release of BSA from the drug-loaded microspheres was checked by UV spectrophotometry (Cary 100 Bio UV Spectrophotometer, Varian) at 595 nm.

Synthesis of star-shaped PLGA

A glass tube was charged with certain amount of D,L-lactide, glycolide, PTOL, and Sn (Oct) $_2$ in dichloromethane (Table 1), which was connected to a vacuum system. Then an exhausting- refilling with nitrogen process was repeated three times. The tube was sealed and put into an oil bath and kept at 160 °C for 8 h. The tube was cooled after the polymerization reaction. The resulting product was dissolved in CH_2Cl_2, precipitated with excess of anhydrous methanol. The purified copolymer was dried in a vacuum oven until constant weight.

Preparation of BSA-loaded microspheres

BSA-loaded microspheres were fabricated by a W/O/W double emulsion solvent evaporation method. A certain amount of BSA was dissolved in water (inner water phase); another amount of PLGA was dissolved in CH_2Cl_2 (oil phase). The two solutions were emulsified for certain time using a sonicator (SONICS & MATERIALS VCX—400). This resulting first emulsion (W/O) was poured into PVA aqueous solution which was hold in ice bath (external water phase) and mixed by mechanical stirring to produce a double emulsion (water-in-oil-in-water). The microspheres were centrifugation at 6,000 rpm for 20 min, washed with deionized water and freeze-dried for 24 h, and then the resultant microspheres were weighted. The amount of BSA contained in the microspheres was obtained by UV spectrophotometry according to standard curve equation. The LC and EE were calculated as follows [25].

Table 1 The ratio of monomers to prepare PLGA

No.	D,L-laclide/glycolide (mol/mol)	Monomers/PTOL (mol/mol)	Monomers/catalyst (mol/mol)
s-PLGA-50/1 (S-1)	3:1	50:1	10,000:1
s-PLGA-100/1 (S-2)	3:1	100:1	10,000:1

$$\text{Loading capacity (LC)}\,(\text{mg/g}) = \text{Content of BSA in microspheres/}\ \text{Qualities of Microspheres for testing} \quad (1)$$

$$\text{Encapsulation efficiency (EE)}\,\% = \left(\text{LC} \times \text{Total qualities of microspheres obtained/}\ \text{Total qualities of BSA for preparation}\right) \times 100\% \quad (2)$$

In vitro drug release

In vitro BSA release of microspheres was carried out in phosphate buffer saline (PBS) with pH 7.4 at 37 °C. A certain amount of BSA-loaded microspheres prepared with different conditions were suspended in PBS buffer. In particular time interval, some microsphere contained solution was taken to be tested.

Results and discussion

The ^{13}C-NMR results of linear-PLGA and star-shaped PLGA

The ^{13}C-NMR results of 1-PLGA, s-PLGA-50/1 and s-PLGA-100/1 are shown in Fig. 1a–c, respectively. As can be seen from these figures, the chemical shift at a ($\delta = 12.27$, 12.32, and 16.85 ppm) were assigned to the C (–CH_3), b ($\delta = 64.75$, 64.84, and 69.18 ppm) were assigned to the C (–CH) and c (56.54, 56.79, and

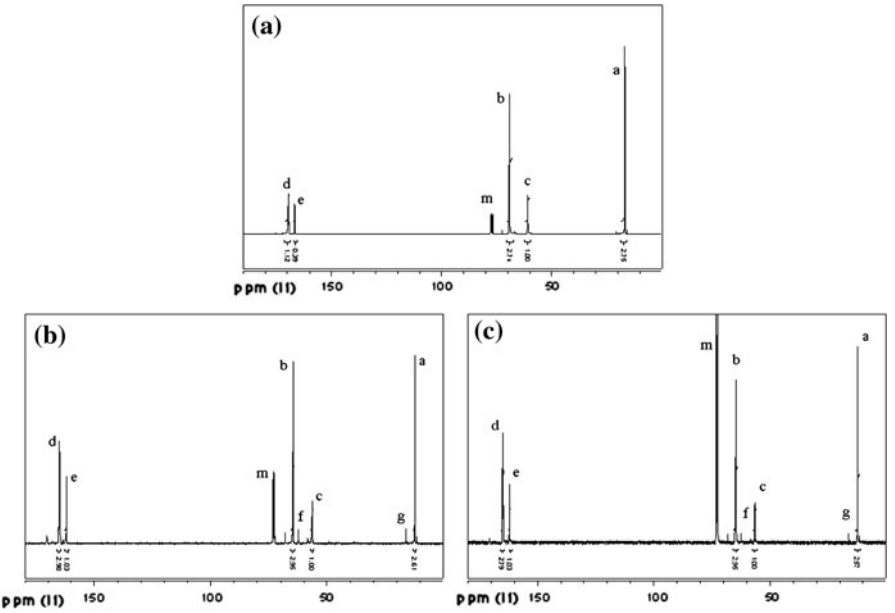

Fig. 1 The ^{13}C-NMR of: **a** 1-PLGA (linear), **b** s-PLGA-50/1 and **c** s-PLGA-100/1

Table 2 The molecular weights of the star-shaped PLGA

No.	M_n	M_w	PDI (M_w/M_n)
s-PLGA-50/1 (S-1)	9,081	10,897	1.20
s-PLGA-100/1 (S-2)	14,032	16,983	1.21

60.86 ppm) were assigned to the C (–CH_2) in the PLGA, respectively. Besides, d ($\delta = 165.26$, 165.21, and 169.60 ppm) and e ($\delta = 162.06$, 162.09, and 166.65 ppm) were assigned to the C (–CO–) in D,L-lactide and glycolide in the PLGA, respectively. And m was solvent peak. However, f ($\delta = 62.25$ and 62.36 ppm) were assigned to the quaternary carbon and g ($\delta = 15.71$ and 16.15 ppm) were assigned to the C (–CH_2) in the PTOL, respectively. These results proved that the star-shaped PLGA was synthesized successfully.

The molecular weights of the star-shaped PLGA

Molecular weights of PLGA, as determined GPC are shown in Table 2. From the Table 2, we knew that the molecular weight of the two star-shaped polymers were approximate 9,000 Da (S-1) and 14,000 Da (S-2), the PDI were 1.20 and 1.21, respectively.

DSC Analysis

The DSC curves of 1-PLGA, s-PLGA-50/1 and s-PLGA-100/1 were shown in Fig. 2. From the figure, it was seen that, the T_g of 1-PLGA was 35.9 °C (M_n: 10,000), however, the T_g of the s-PLGA-50/1 which the molecular weight (M_n: 9,081) was close to 1-PLGA that was 39.3 °C, it was higher than the T_g of 1-PLGA.

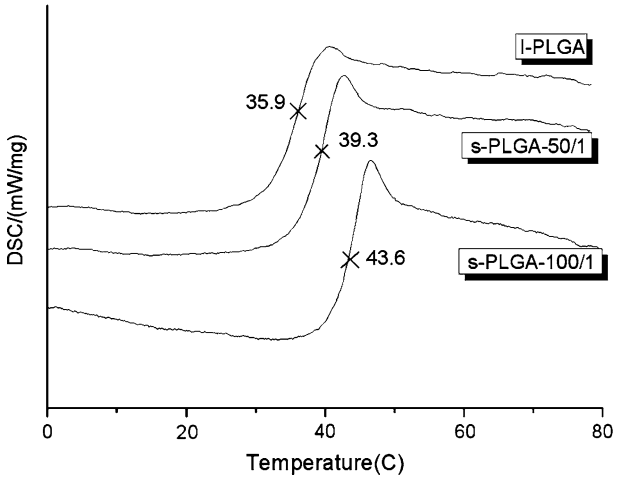

Fig. 2 The DSC curves (1-PLGA (linear), s-PLGA-50/1 and s-PLGA-100/1)

It was because that the branching structure will reduce the flexibility of the molecular chain of the polymer, the T_g of the polymer would increase. The T_g of s-PLGA-100/1 was 43.6 °C (M_n: 14,032), it was higher than the T_g of s-PLGA-50/1 because of the higher molecular weight.

Loading capacity and encapsulation efficiency (EE%) of BSA-loaded microspheres with different polymers

LC and EE% of BSA-loaded microspheres with different polymers were shown in Table 3. LC and EE% of microspheres fabricated from star-shaped PLGA were higher than that of linear PLGA at the same amount, the reasons are, on the one hand, many longer arms of star-shaped PLGA could interact with BSA molecule from various directions, which exhibit higher efficiency than the linear PLGA, on the other hand, the quantity of the terminal hydroxyl group in the star-shaped PLGA was more than in the linear PLGA, the interaction between the terminal hydroxyl group and BSA caused by hydrogen bond is stronger in the star-shaped PLGA, so the amount of BSA encapsulated into the microspheres was increased. For different star-shaped PLGA, as molecular weight increased, each arm was relatively longer, both LC and EE% were increased. For the same amounts of BSA, the EE% was increased with the increasing of the PLGA used in the encapsulation, but the LC was decreased. All of the LC and EE% from s-PLGA are better than l-PLGA.

Loading capacity and encapsulation efficiency of BSA-loaded microspheres with star-shaped PLGA fabricated in different conditions

Effect of the preparation conditions of star-shaped PLGA microspheres on LC and EE% was shown in Table 4. The mechanical stirring rate influenced the LC and EE% greatly. Within the range of the experimental condition, for S-2-2-1 and S-2-2-2, as the stirring rate was decreased from 300 to 200 r/m when first emulsion diffused into external water phase, the LC was decreased from 52.28 to

Table 3 Loading capacity and encapsulation efficiency of BSA-loaded microspheres with different polymers

No.	Molecular weight of PLGA (kDa)	Kinds of PLGA in oil phase	Weight of PLGA in oil phase (g)	LC (μg/mg microspheres)	EE (%)
S-1-1	9	s-PLGA-50/1	0.6	37.85	31.90
S-1-2	9	s-PLGA-50/1	0.8	31.18	33.41
S-1-3	9	s-PLGA-50/1	1.0	28.88	49.10
S-2-1	14	s-PLGA-100/1	0.6	67.51	61.72
S-2-2	14	s-PLGA-100/1	0.8	52.28	61.99
S-2-3	14	s-PLGA-100/1	1.0	49.17	70.24
L-1	10	l-PLGA	0.6	11.48	9.68
L-2	10	l-PLGA	0.8	9.98	10.98
L-3	10	l-PLGA	1.0	9.48	12.47

Table 4 Loading capacity and encapsulation efficiency of BSA-loaded microspheres with star-shaped PLGA fabricated in different conditions

No.	Mechanic stirring rate[a] (*r*/min)	Ultrasonic time[b] (s)	Volume of inner water phase (mL)	Volume of DCM in oil phase (mL)	NaCl in external water phase (mg/mL)	NaCl in inner water phase (mg/mL)	LC (μg/mg microspheres)	EE (%)
S-2-2-1	300	30	0.3	12	0	0	52.28	61.99
S-2-2-2	200	30	0.3	12	0	0	39.35	44.94
S-2-2-3	400	30	0.3	12	0	0	66.92	78.39
S-2-2-4	300	120	0.3	12	0	0	59.82	70.93
S-2-2-5	300	30	1.2	12	0	0	61.38	70.15
S-2-2-6	300	30	0.3	12	2.5	0	61.93	73.43
S-2-2-7	300	30	0.3	12	0	5	31.65	35.27
S-2-2-8	300	30	0.3	20	0	0	48.94	55.93

[a] The mechanic stirring was used when first emulsion diffused into external water phase

[b] Ultrason was used in the preparation of first emulsion

39.35 μg/(mg microspheres) and the EE% was decreased from 61.99 to 44.94%; and then the stirring rate for S-2-2-3 was increased to 400 *r*/m, the LC and EE% were increased to 66.92 μg/(mg microspheres) and 78.39%, respectively, compared to S-2-2-1. For S-2-2-4, when the ultrasonic time for produce first emulsion increased from 30 to 120 s, the LC and EE% were slightly increased. For S-2-2-5 and S-2-2-6, while the relative volume of oil phase was increased from 0.3 to 1.2 or the addition of the salt to the external water phase, the LC and EE% were increased in varying degrees. However, for S-2-2-8, when the volume of DCM in oil phase was increased from 12 to 20, the LC and the EE% were decreased slightly. For S-2-2-7, when the addition of salt to the inner water phase, the LC and EE% were decreased greatly to 31.65 μg/(mg microspheres) and 35.27%, respectively. The maximum LC and EE% were 66.92 μg/(mg microspheres) and 78.39%, respectively, in this experimental conditions.

SEM micrograph of BSA-loaded microspheres before and after drug release

Figure 3 showed the SEM micrograph of BSA-loaded microspheres before and after drug release. It was clear that the BSA-loaded microspheres were regular spherical in shape and 80–150 μm in diameter. The microspheres were not adhesive, but the surface of the microspheres was not very smooth. It was also clearly seen the collapse state of microspheres after 37 h due to the degradation and deformation of the polymer.

The release curves of S-1-2 and S-2-2 microspheres

The release curves of S-1-2 and S-2-2 microspheres were shown in Fig. 4. The in vitro release profiles of BSA lasted for 37 h. For the drug-loaded microspheres

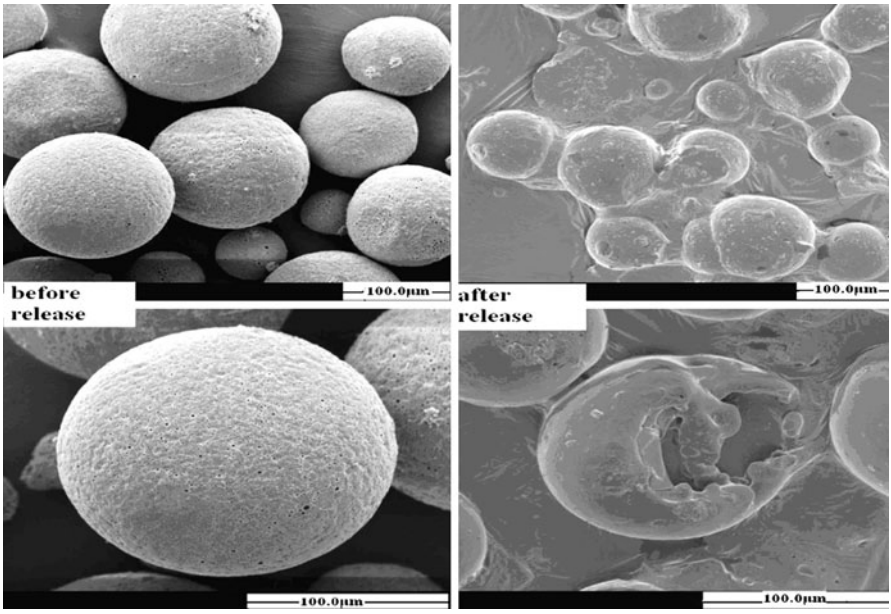

Fig. 3 SEM micrograph of BSA-loaded star-shaped PLGA microspheres before and after drug release

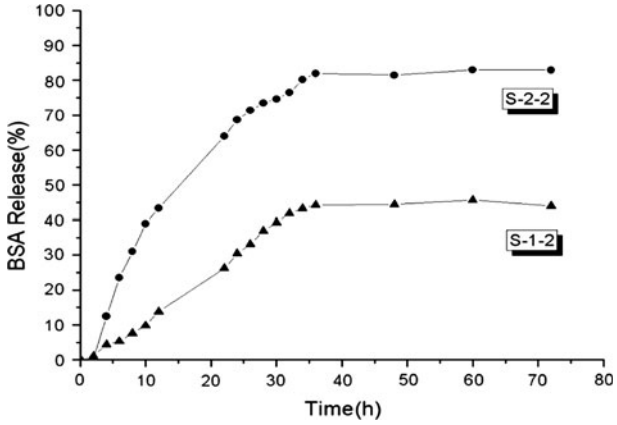

Fig. 4 Release curves of S-1-2 and S-2-2 microspheres

fabricated from star-shaped PLGA with different molecular weight at the same amount, the microspheres made from higher molecular weight PLGA (S-2-2) exhibit a more complete release. This can be attributed to the fact that the LC of the microspheres made from the higher molecular weight of PLGA (S-2-2) was more than that of the lower molecular weight PLGA(S-1-2). During the course of the drug release the more burst release phenomena could be observed. In addition, for the lower molecular weight PLGA, more degradation of PLGA resulting in the stronger acidic microenvironment, which caused a stronger trend of BSA aggregation [26],

so the release was not complete, only about 40%. While the release of microspheres made from PLGA with higher molecular weight was more than 80%.

Conclusions

We have prepared a new star-shaped PLGA copolymer and microspheres of the star-shaped PLGA as delivery carrier for BSA-mode drugs. Various preparing parameters, including weight of PLGA in oil phase, the type of PLGA in oil phase, volume ratio of inner water phase to oil phase, volume ratio of primary emulsion to external water phase, and stirring mode were altered during the microspheres production. The effect of these changes on the microspheres preparation, drug release LC and EE% were determined and optimized. The results showed that the structure and the molecular weight of PLGA were controlled by initiator PTOL. The results indicated that the T_g, LC, and EE% of star-shaped PLGA were higher than that of linear PLGA. The controlled release characteristics of the microspheres for BSA were investigated in pH 7.4 media. The results indicated that drug-loaded microspheres fabricated from star-shaped PLGA exhibit full release, the maximum release percentage was up to 80%. Moreover, the release behavior of microspheres showed the feasibility of BSA-loaded microspheres as controlled release devices.

Acknowledgment We are grateful the financial support of Tianjin Science and Technology Key grants (05YFGPGX26200) and the NSFC of China grants 50873114.

References

1. Kost J, Langer R (2001) Responsive polymeric delivery systems. Adv Drug Deliv Rev 46:125–148
2. Wei L, Cai CH, Lin JP, Chen T (2009) Dual-drug delivery system based on hydrogel/micelle composites. Biomaterials 30:2606–2613
3. Ito F, Fujimori H, Honnami H, Kawakami H, Kanamura K, Makino K (2009) Study of types and mixture ratio of organic solvent used to dissolve polymers for preparation of drug-containing PLGA microspheres. Eur Polym J 45:658–667
4. Fundueanu G, Constantin M, Ascenzi P (2008) Preparation and characterization of pH- and temperature-sensitive pullulan microspheres for controlled release of drugs. Biomaterials 29:2767–2775
5. Ghahremankhani AA, Dorkoosh F, Dinarvand R (2007) PLGA-PEG-PLGA tri-block copolymers as an in situ gel forming system for calcitonin delivery. Polym Bull 59:637–646
6. Wang YC, Liu XQ, Sun TM, Xiong MH, Wang J (2008) Functionalized micelles from block copolymer of polyphosphoester and poly(ε-caprolactone) for receptor-mediated drug delivery. J Control Release 128:32–40
7. Sah H, Lee BJ (2006) Development of new microencapsulation techniques useful for the preparation of PLGA microspheres. Macromol Rapid Commun 27:1845–1851
8. Langer R (2000) Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res 33:94–101
9. Cohen H, Levy RJ, Gao J, Fishbein I, Kousaev V, Sosnowski S, Golomb G (2000) Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. Gene Ther 7:1896–1905
10. Jin C, Bai L, Wu H, Song WJ, Guo GZ, Dou KF (2009) Cytotoxicity of paclitaxel incorporated in PLGA nanoparticles on hypoxic human tumor cells. Pharm Res 26:1776–1784
11. Wang Z, Chui WK, Ho PC (2009) Design of a multifunctional PLGA nanoparticulate drug delivery system: evaluation of its physicochemical properties and anticancer activity to malignant cancer cells. Pharm Res 26:1162–1171

12. Erden N, Celebi N (1996) Factors influencing release of salbutamol sulphate from poly(lactide-*co*-glycolide) microspheres prepared by water-in-oil-in-water emulsion technique. Int J Pharm 137:57–66
13. Lu DD, Yuan JC, Li HG, Lei ZQ (2008) Synthesis and characterization of a series of biodegradable and biocompatible PEG-supported poly(lactic-ran-glycolic acid) amphiphilic barbell-like copolymers. J Polym Sci A 46:3802–3812
14. Lee SJ, Han BR, Park SY, Han DK, Kim SC (2006) Sol–gel transition behavior of biodegradable three-arm and four-arm star-shaped PLGA-PEG block copolymer aqueous solution. J Polym Sci A 44:888–899
15. Dong CM, Qiu KY, Gu ZW et al (2002) Synthesis of star-shaped poly(D,L-lactic acid-alt-glycolic acid)-b-poly(L-lactic acid) with the poly(D,L-lactic acid-alt-glycolic acid) macroinitiator and stannous octoate catalyst. J Polym Sci 40:409–415
16. Dong CM, Qiu KY, Gu ZW, Feng XD (2001) Synthesis of star-shaped poly(D,L-lactic acid-alt-glycolic acid) with multifunctional initiator and SnOct2 catalyst. Polymer 42:6891–6896
17. Dong CM, Qiu KY, Gu ZW, Feng XD (2001) Synthesis of star-shaped poly(ε-caprolactone)-*b*-poly(DL-lactic acid-*alt*-glycolic acid) with multifunctional initiator and stannous octoate catalyst. Macromolecules 34:4691–4696
18. Meng FT, Ma GH, Liu YD, Qiu W, Su ZG (2004) Microencapsulation of bovine hemoglobin with high bio-activity and high entrapment efficiency using a W/O/W double emulsion technique. Colloids Surf B 33:177–183
19. Benichou A, Aserin A, Garti N (2007) W/O/W double emulsions stabilized with WPI-polysaccharide complexes. Colloids Surfaces A 294:20–32
20. Jain RA (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-*co*-glycolide) (PLGA) devices. Biomaterials 21:2475–2490
21. Gvili K, Benny O, Danino D, Machluf M (2007) Poly(D,L-lactide-*co*-glycolide acid) nanoparticles for DNA delivery: waiving preparation complexity and increasing efficiency. Biopolymers 85:379–391
22. Freitas S, Merkle HP, Gander B (2005) Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. J Control Release 102:313–332
23. Takada S, Yamagata Y, Misaki M, Taira K, Kurokawa T (2003) Sustained release of human growth hormone from microcapsules prepared by a solvent evaporation technique. J Control Release 88:229–242
24. Zhang ZP, Liu Q, Liu MM, Wu XD (2009) Synthesis and characterization of star-shaped poly (lactide-*co*-glycolide) and its drug-loaded microspheres. CN1927906
25. Nagaich S, Khopade AJ, Jain NK (1999) Lipid grafts of egg-box complex: a new supramolecular biovector for 5-fluorouracil delivery. Pharm Acta Helv 73:227–236
26. Liu SQ, Yang YY, Liu XM, Tong YW (2003) Preparation and characterization of temperature-sensitive poly(*N*-isopropylacrylamide)-b-poly(D,L-lactide) microspheres for protein delivery. Biomacromolecules 4:1784–1793