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Preparation of chitosan–gelatin scaffold containing tetrandrine-loaded nano-aggregates and its controlled release behavior

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Abstract

A well-timed delivery of bioactive macromolecules from the porous scaffolds is very important in tissue engineering. Tetrandrine (Ted) is one of a large number of known plant derived bisbenzylisoquinoline alkaloids and is obtained from the roots of *Stephania tetrandria*. Ted can be used as a modifier to poly(L-lactic acid) scaffolds to promote chondrocyte differentiation and secrete type II collagen. But the effect of Ted on chondrocyte's behavior strongly depends on the concentration of Ted in the culture media. Here amphiphilic diblock copolymer (PLAE) composed of L-lactide and methoxy poly(ethylene glycol) (MePEG) was prepared and the Ted loaded copolymeric nanospheres were obtained by self-emulsification and then solvent evaporation. The mean sizes of core/shell type PLAE nanospheres and Ted-loaded nanospheres are about 60 and 100 nm, respectively. Chitosan–gelatin (Cs–Gel) porous scaffolds loaded with PLAE–Ted nanospheres were fabricated through freeze drying. Ted release behaviors from PLAE–Ted nanospheres and porous scaffolds were investigated. The result shows that the Ted-loaded nanospheres could be embedded within Cs–Gel scaffolds and no initial burst release could be observed in the release patterns. Here a room can be provided for the scaffolds to sustained release bioactive component in tissue engineering.

Keywords: PLA/MePEG diblock copolymer; Nanosphere; Scaffolds; Drug release; Tetrandrine

1. Introduction

The repair of damaged or worn out tissues is an increasing concern. By combining different approaches taken from biotechnology, biology and material science, tissue engineering aims to provide efficient tools to reach this goal (Griffith and Naughton, 2002). An approach in this field consists of combining porous supportive structures with bioactive molecules to guide the tissue regeneration more efficiently. A well-timed delivery of the bioactive compounds from the scaffold is necessary to reach the desired effect (Vasita and Katti, 2006; Zisch et al., 2003).

Many methods of drug delivery from scaffolds have been developed for use in engineering the different tissues. The method of drug delivery should be selected based on the application, scaffold material and target drug. One of the most common methods of creating controlled release is to utilize the physical properties of the scaffold material to regulate the amount of drug delivered. To create controlled release, the target drug is mixed with the scaffold precursors during fabrication. In such systems, the properties of the scaffold, such as pore size or crosslinking density, could regulate the drug release rate by diffusion. The rate of scaffold degradation also can affect how much drug is released over time (Tachibana et al., 2006). Another method of creating controlled release is to incorporating drugs into microspheres (Benoit et al., 2000). The release kinetics of the target drug can be altered by changing the polymer used as carrier, the amount of drug loaded and the size of the microsphere (DeFail et al., 2006). The microspheres can either be injected directly into the desired location or incorporated into scaffolds made from other materials. In the later case, the scaffolds are made of different materials that contain pores large enough to allow

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axons and/or cells to migrate into the scaffolds (Goraltchouk et al., 2006). Recently, self-assemblies or aggregation of polymeric amphiphiles in aqueous media have been studied with respect to biological importance and pharmaceutical application. There has been an increasing interest in the development of drug delivery systems by using amphiphilic copolymer derived from polylactide and poly(ethylene glycol) (PEG) as carriers (Li et al., 2005; Liggins and Burt, 2002). Some micelles of poly(lactide-*b*-ethylene glycol) loaded drug, e.g. Taxol (Genexo-PM) have been available commercially (Kim et al., 2001; Liu et al., 2001).

The herb components have multiple biological activities. Some of them have showed the tissue regeneration potentials (Ikezoe et al., 2002; Mundy et al., 1999; Niizawa et al., 2003; Suk et al., 2003). Therefore, their delivery systems will provide chemical cues in tissue engineering. However, these components usually exhibit poor aqueous solubility, rendering delivery of these compounds quite challenging. Tetrandrine (Ted) is one of a large number of known plant derived bisbenzylisoquinoline alkaloids and is obtained from the roots of *Stephania tetrandria*. It possesses anti-inflammatory, antiplatelet aggregation, Ca^{2+} channel block, immunosuppressive and free radical scavenging effects (Ho and Lai, 2004; Kim et al., 1999; Lai et al., 1999; Wang et al., 2004), so it has been used as an analgesic, diuretic and anti-inflammatory agent in China (Lai, 2002). Many recent studies reported the influences of Ted to the behaviors of different kinds of cells (Fu et al., 2002). Ted can also be used as a modifier to poly(lactic acid) scaffold to promote chondrocytes differentiation to secrete Type II collagen (Cui et al., 2005). A chitosan–gelatin (Cs–Gel) scaffold has promising perspective in cartilage tissue engineering (Xia et al., 2004).

Based on these opinions, here we constituted a Ted-loaded nanosphere embedded in Cs–Gel scaffolds. Firstly, nano-aggregates of poly(L-lactide)-*b*-poly(methoxy ethylene glycol) (PLA/MePEG diblock copolymer, PLAE) were prepared. The resulting core/shell nanospheres could be used to encapsulate Ted. Then PLAE copolymer micelles uploaded with Ted were embedded in chitosan–gelatin scaffolds and the controlled released behaviors of Ted from the scaffolds were investigated.

2. Experimental

2.1. Materials

L-Lactide was prepared from L-lactic acid (Purac Chemicals, Netherlands) and recrystallized from ethyl acetate by our lab. Methoxy poly(ethylene glycol) with molecular weight 2000 was purchased from Fluka. Stannous octoate was obtained from Sinica Medicine Group Chemical. Chitosan (Cs) powder (molecular weight 1.0×10^5, degree of deacetylation being 85%) was provided by Haihui Bioengineering Ltd. Co. (Qingdao, China). Gelatin (Gel, mean average molecular weight 1.0×10^5) was purchased from Sigma Chemical Co. Tetrandrine (Ted) (cf. Fig. 1) was purchased from National Institute for the Control of Pharmaceutical and Biological Products.

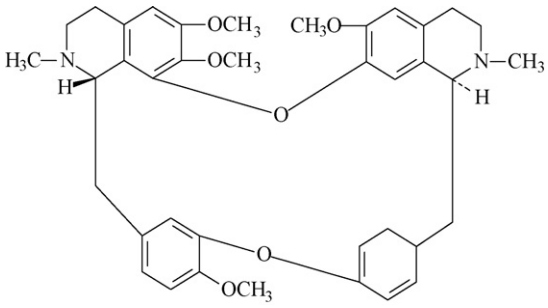

Fig. 1. Structural formula of tetrandrine.

2.2. Preparation and characterization of PLAE diblock copolymer

2.2.1. Preparation of PLAE diblock copolymer

PLA/MePEG (PLAE) diblock copolymer was synthesized by ring-opening polymerization of lactide with MePEG in the presence of stannous octoate as a catalyst at 140 °C for 36 h in a vacuum (Huang et al., 1999). Prior to the polymerization, lactide was recrystallized from ethyl acetate and washed with hot diethyl ether. MePEG was purified by precipitation from an acetone solution into petroleum ether. The mole ratio of $Sn(Oct)_2$ to MePEG was 4.16×10^{-3}. The obtained copolymers were dissolved in acetone and subsequently precipitated with excess diethyl ether at room temperature. The polymer was then dried at 40 °C in a vacuum for 48 h.

2.2.2. Characterization of PLAE copolymer

PLAE copolymer was analyzed by ^{1}H NMR, GPC. ^{1}H NMR spectra were recorded on a Varian Mercury Vx300 300 HZ spectrometer using deuterated chloroform as a solvent. The GPC system consisted of a Waters Alliance 2690 separation module, a Waters 484 tunable absorbance detector, an on-line multiangle laser light scattering (MALLS) detector (MiniDawn, Wyatt Technology Inc.), an interferometric refractometer (Optilab DSP, Wyatt Technology Inc.), and two 3 lm PLgel (Polymer Laboratories Inc.) columns connected in series. The system used THF as the mobile phase at a flow rate of 1 mL/min, and sample concentrations ranged from 7 to 10 mg/mL.

2.3. Preparation and characterization of PLAE and PLAE–Ted nanospheres

2.3.1. Preparation of PLAE and PLAE–Ted nanospheres

PLAE nanospheres were prepared via self-emulsifying and solvent-evaporating method. Briefly, PLAE copolymer (150 mg) was dissolved into 3 mL methylene dichloride. The solution obtained was added into 15 mL ultra-purified water and agitated overnight. Nanospheres were formed along with organic solvent evaporation. Nanosphere dispersion was obtained after filtration with a 0.45 μm filtered membrane (Millipore, USA). As for PLAE–Ted nanospheres, the only different was adding a certain amount of Ted into the PLAE copolymer CH_2Cl_2 solution. The other processes were exactly same as that for PLAE nanospheres.

2.3.2. Determination of size and size distribution of nanospheres

To determine the size and size distribution of PLAE or PLAE–Ted nanospheres, dynamic light scattering (DLS) measurements were carried out using a dynamic light scattering spectrometer (Model BI9000AT, Brookhaven, USA) at a wavelength of 514 nm at 20 °C. The intensity of the scattered light was detected at 90° to the incident beam. An average size distribution of nanospheres was determined based on CONTIN programs of Provencher et al.

2.3.3. Morphologies analysis of nanospheres

The sizes and shapes of nanospheres were observed with a TEM (Model TEM-100CXII JEOL, Japan). A drop of solution containing nanospheres was deposited on the grid and after the excess has been taken off, a drop of the negative staining solution (phosphotungstic acid) was then deposited on the grid. After a few seconds the excess fluid was drain off by applying a narrow tapered strip of filter paper to the edge of the grid and the grid was allowed to dry before observation with the TEM.

2.4. Preparation and characterization of Cs–Gel scaffolds containing nanospheres

2.4.1. Preparation of Cs–Gel scaffolds containing PLAE–Ted nanospheres

The chitosan–gelatin (Cs–Gel) network scaffolds were fabricated according the known method (Mao et al., 2003). Chitosan (Cs, 0.2 g) was dissolved in 10 mL 2% (w/w) acetic acid aqueous solution containing 0.4 g gelatin (Gel) at 37 °C under agitating for 12 h. Then 0.7 mL 0.25% (w/w) glutaraldehyde aqueous solution was added. Afterward, PLAE–Ted nanosphere suspension was added and continued stirring for 10 min. After degassing, the mixture was cast into a model for further freezing at −50 °C for 12 h and lyophilizing for 36 h to yield the PLAE–Ted nanospheres embedded chitosan–gelatin scaffolds (Cs–Gel–PLAE–Ted). As control, Cs–Gel–Ted scaffolds were prepared as the same, only Ted was added in the Cs–Gel acetic acid aqueous solution directly not in the form of PLAE–Ted nanospheres.

2.4.2. Morphologies of Cs–Gel–PLAE–Ted scaffolds

To observe the morphologies of the scaffolds, the specimens were coated with gold–palladium and examined under a scanning electron microscope. PhlilpsXL30 environment scanning electron microscope was used.

2.4.3. Determination of scaffold porosity

The porosity was measured by liquid substitution method. The liquid used in this study was isopropanol. The porosity ε was calculated as follow:

$$\varepsilon = \frac{V_1 - V_3}{V_2 - V_1}$$

where V_1 is the volume of isopropanol before the scaffold was put in; V_2 the volume of the liquid after the scaffold was put in; V_3 is the volume of isopropanol after the liquid was pressed into the pore of the scaffold and the scaffold was taken out of the liquid. Eight specimens of each sample were tested. The average and standard deviations were presented.

2.4.4. Mechanical analysis

For the characterization of mechanical properties, the porous scaffolds were compressively tested to record the stress–strain curves. The tests were performed using a Testmetric M350-25 KN materials testing system at room temperature. A testing rate, that is, a crosshead speed was set as 1 mm/min. Cylindrical specimens with dimensions of 10 mm in height and 10 mm in diameter were used for the tests. Five specimens of each sample were tested. The average and standard deviations were presented.

2.5. Determination of Ted release behaviors

The PLAE–Ted nanospheres, Cs–Gel–Ted or Cs–Gel–PLAE–Ted scaffolds were put into a dialysis bag containing 5 mL PBS buffered solution (pH 7.4) against 250 mL PBS buffered solution at 37 °C. At predetermined time intervals, 1.0 mL of released solution was withdrawn for measuring, which was replenished by 1.0 mL of fresh PBS solution to maintain a constant volume. The concentration of Ted released was monitored, using a UV detector at 283 nm provided by a HPLC (Aglient 1100) equipped with Kromasil C18 250 mm × 4.6 mm, 5 μm column and methanol–water–triethylamine (85:15:0.25 by volume) solution as a flow phase at 25 °C and flow rate of 1 mL/min. Three specimens of each sample were tested. The average and standard deviations were presented.

3. Results and discussion

3.1. Synthesis of PLAE diblock copolymer

PLAE copolymer was synthesized by a ring opening polymerization of L-lactide in the presence of MePEG prepolymer using stannous octoate as a catalyst. The composition and molecular weight of PLAE copolymer were evaluated by ^{1}H NMR spectroscopy and GPC measurement. Fig. 2 shows the ^{1}H NMR spectrum of PLAE copolymer. The peaks at 5.18 ppm (CH) and 1.60 ppm (CH_3) belong to PLA blocks and the peak at 3.56 ppm is the characteristic of main-chain methylene units in PEG blocks. The signal at 3.38 ppm can be attributed to the three chemically equivalent hydrogen atoms of the methyl group at the end of the MePEG block in the polymer chains. Its integral serves as an internal standard to calculate the number average chain length of the MePEG. The unit ratio of MePEG and lactide could be obtained from the peak intensities of the methylene proton (δ = 3.56 ppm) of the ethylene in MePEG, and the methylidyne proton (δ = 5.18 ppm) in lactide segments. While the number average molecular weight of PLAE diblock copolymer can also be calculated. The number average molecular weight of PLAE here used for the preparation of PLAE nanospheres is 4850 (^{1}H NMR), molar ratio of MePEG to lactic acid unit is 1:20 (^{1}H NMR) and the molecular weight polydispersity Mw/Mn = 1.04 (GPC).

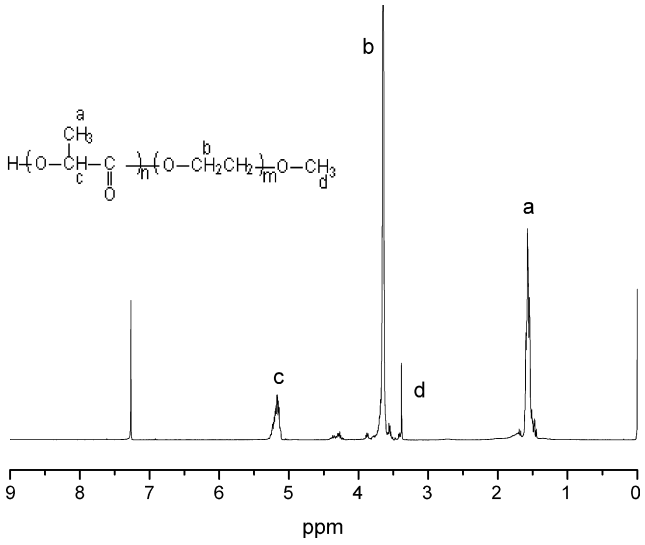

Fig. 2. ^{1}H NMR spectrum of PLAE diblock copolymer in $CDCl_3$.

3.2. Formation of PLAE nanospheres

In the past decades, polymeric micelles prepared from amphiphilic block copolymers have been largely investigated for sustained delivery of drugs (Gaucher et al., 2005). These copolymers can form micelle-like structure with a core–shell structure through auto-assembly in aqueous media: the hydrophobic segments aggregate to form an inner core able to accommodate hydrophobic drugs with improved solubility; the hydrophilic shell consists of a brush-like protective corona that stabilizes the micelles in aqueous solution. The PLAE block copolymer is susceptible to form micelles in aqueous media. The hydrophobic core of the micelles consists of bioresorbable PLA segments, and the hydrophilic shell of a PEG corona. The size and size polydispersity of the obtained PLAE and PLAE–Ted nanospheres were measured by DLS and summarized in Table 1. The mean diameter of the PLAE nanospheres is about 60 nm. The polydispersity factor of the micelles is fairly low, suggesting a narrow size distribution. The diameters of PLAE–Ted nanospheres are significantly bigger than PLAE. Ted is a lipophilic compound (cf. Fig. 1), and it can be solubilized in the hydrophobic interior of PLAE nanospheres. So the volume of the core of core/shell PLAE–Ted nanospheres would become larger when the loaded drug amount increased.

Table 1
Size and size polydispersity of PLAE and PLAE–Ted nanospheres

Samples	Diameter (nm)	Polydispersity (μ/Γ^2)
PLAE	56.4	0.190
PLAE–Ted (5)*	82.1	0.223
PLAE–Ted (10)	95.5	0.165
PLAE–Ted (20)	110.2	0.196

PLAE–Ted (5) is Ted-loaded nanosphere and the Ted addition percentage in the sphere is 5%.

In order to characterize the morphology of PLAE and PLAE–Ted nanospheres, TEM measurement was carried out. Fig. 3 shows the photographs of PLAE and PLAE–Ted nanospheres under TEM observation. It could be seen that most of the nanospheres have a regular spherical shape and the size is in the range 40–100 nm. By close observation of TEM photographs, it could be found that bright and dark regions were seen in these nanoparticles. The bright region should respond to the PEG block and the dark region should be attributed to the

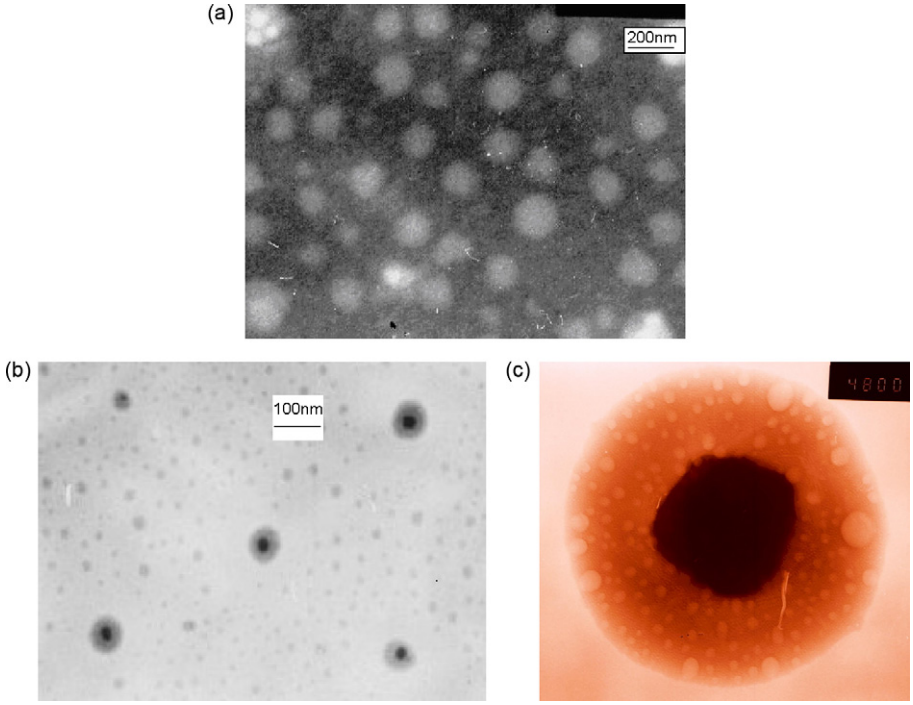

Fig. 3. TEM micrographs of PLAE and PLAE–Ted nanospheres staining with tungsten phosphate. *(a) PLAE diblock copolymer nanospheres; (b and c) Ted-loaded nanospheres PLAE–Ted (20).

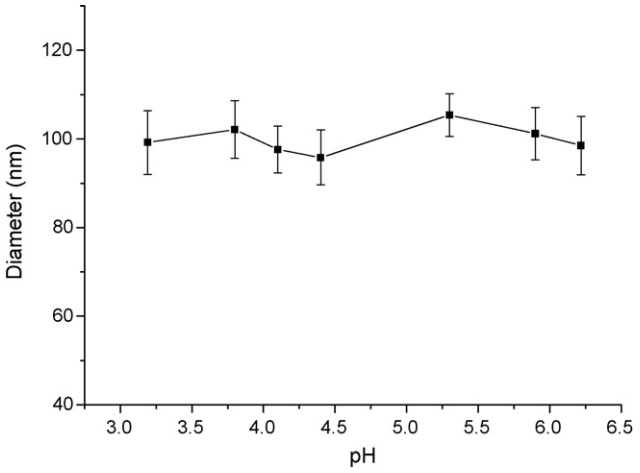

Fig. 4. Variation of micellar size of PLAE–Ted (10) in buffered solutions with different pH values.

hydrophobic block of PLA and Ted. Hydrophobic Ted is encapsulated in the core part of the nanospheres and their core/shell type structure could be observed much more obviously.

3.3. Preparation and characterization of Cs–Gel–PLAE–Ted porous scaffolds

Because chitosan can only be dissolved in acidic solution, chitosan and gelatin acetic acid aqueous solution was used as the Cs–Gel porous scaffolds precursor. In order to combine Cs–Gel scaffolds with PLAE–Ted nanospheres, the fabrication process must be carried out in an acidic solution. So the stability of PLAE–Ted nanospheres in the acidic solution should play an important role. Fig. 4 shows the relationship between the diameter of PLAE–Ted nanospheres and pH value of the dispersing solution.

Because there is no ionic group on the molecular chain of PLAE, the size of PLAE–Ted nanospheres exhibits good pH stability. That will be benefit for embedding the nanospheres into some scaffolds in tissue engineering when the scaffolds must be fabricated in different acidic or basic medium.

3.3.1. Porosities of scaffolds

A porous Cs–Gel scaffold was fabricated through four main procedures including Cs–Gel polyelectrolyte complex hydrogel formation, swelling, lyophilization with water in the network as a porogen and crosslinking. Its porosity depends mainly on the concentration of the original polymers in the acetic acid aqueous solution. Moreover, pore size and characters relate to the heat transfer performance. The mean pore size of the scaffolds obtained here varies from approximately 150–250 μm, and it can be controlled by the freezen temperature (Mao et al., 2003). The freezing and lyophilizing process would generate in an open microstructure with a high degree of interconnectivity. The pore sizes of the obtained scaffolds are fit for cell in growth and angiogenesis after transplantation. From Table 2 it can be seen that the porosity of Cs–Gel–PLAE scaffold is significantly higher than that of Cs–Gel scaffold. PLAE was introduced to Cs–Gel acetic acid aqueous solution in the form of nanosphere suspension. That resulted in the decreasing of the concentration of Cs–Gel network in Cs–Gel–PLAE scaffold's fabrication precursor solution compared with Cs–Gel scaffold. On the other hand, the increasing of water (playing the role of porogen) during the formation of the porous scaffolds, made the porosity of Cs–Gel–PLAE scaffolds relatively higher. Because the dosage of Ted was very little compared with Cs–Gel or Cs–Gel–PLAE solution, it could hardly take any effects on the porosity of the scaffolds.

Table 2
Porosity of the scaffolds

Code	Scaffold	PLAE (wt%)	Ted (wt%)	Porosity (%)
Scaffold-1	Cs–Gel	0	0	93.23 ± 0.32
Scaffold-2	Cs–Gel–Ted	0	0.21	93.24 ± 0.26
Scaffold-3	Cs–Gel–Ted	0	1.23	93.04 ± 0.45
Scaffold-4	Cs–Gel–Ted	0	1.93	93.32 ± 0.28
Scaffold-5	Cs–Gel–Ted–PLAE	2.44	0	95.41 ± 0.25
Scaffold-6	Cs–Gel–Ted–PLAE	4.92	0	96.05 ± 0.31
Scaffold-7	Cs–Gel–Ted–PLAE	6.97	0	96.68 ± 0.36
Scaffold-8	Cs–Gel–Ted–PLAE	9.09	0	97.25 ± 0.24
Scaffold-9	Cs–Gel–Ted–PLAE–Ted	4.92	0.20	96.35 ± 0.26
Scaffold-10	Cs–Gel–Ted–PLAE–Ted	4.92	1.23	96.04 ± 0.32
Scaffold-11	Cs–Gel–Ted–PLAE–Ted	4.92	1.92	96.11 ± 0.34

3.3.2. Morphologies of scaffolds

Fig. 5 shows the morphologies of Cs–Gel–Ted and Cs–Gel–PLAE–Ted scaffolds. Compared with Cs–Gel–Ted scaffold, the adding of PLAE–Ted nanospheres made the pore size of the Cs–Gel–PLAE–Ted scaffolds more homogeneously, and also resulted in the increasing of the porosity (Table 2). That may be because PLAE could play as a kind of surfactant and ice crystal nuclei. The existence of PLAE resulting in the porogen distributing more homogeneously and the amount of initially formed ice crystal nuclei enhanced.

As for the distribution of PLAE–Ted nanospheres in the scaffolds, c and d in Fig. 5 shows that the nanospheres have been well embedded in the scaffolds and distributed evenly. The sizes of the particles on the wall surface of the pores reflected that some of PLAE–Ted nanospheres aggregated in the fabrication process of Cs–Gel–PLAE–Ted scaffolds.

3.3.3. Mechanical properties of scaffolds

During cell cultivation, 3D porous scaffolds need to have appropriate strength for cell attachment. Fig. 6 shows the compressive stress–strain curves of Cs–Gel and Cs–Gel–PLAE scaffolds. From the diagram, we could see that Cs–Gel scaffold has stronger mechanical strength than Cs–Gel–PLAE scaffolds (cf. Table 3). The interactions, such as hydrogen bond, between chitosan and gelatin or themselves would decline because of the joining of PLAE. So the bulk strength of Cs–Gel network should be higher than that of the blend composed of Cs–Gel complex and PLAE. So the more the PLAE was introduced, the lower the compressive strength of Cs–Gel–PLAE scaffold was. On the other hand, Cs–Gel scaffold has a relatively lower porosity than Cs–Gel–PLAE scaffolds (cf. Table 2), that also resulting in the Cs–Gel scaffold could withstand stronger compression force.

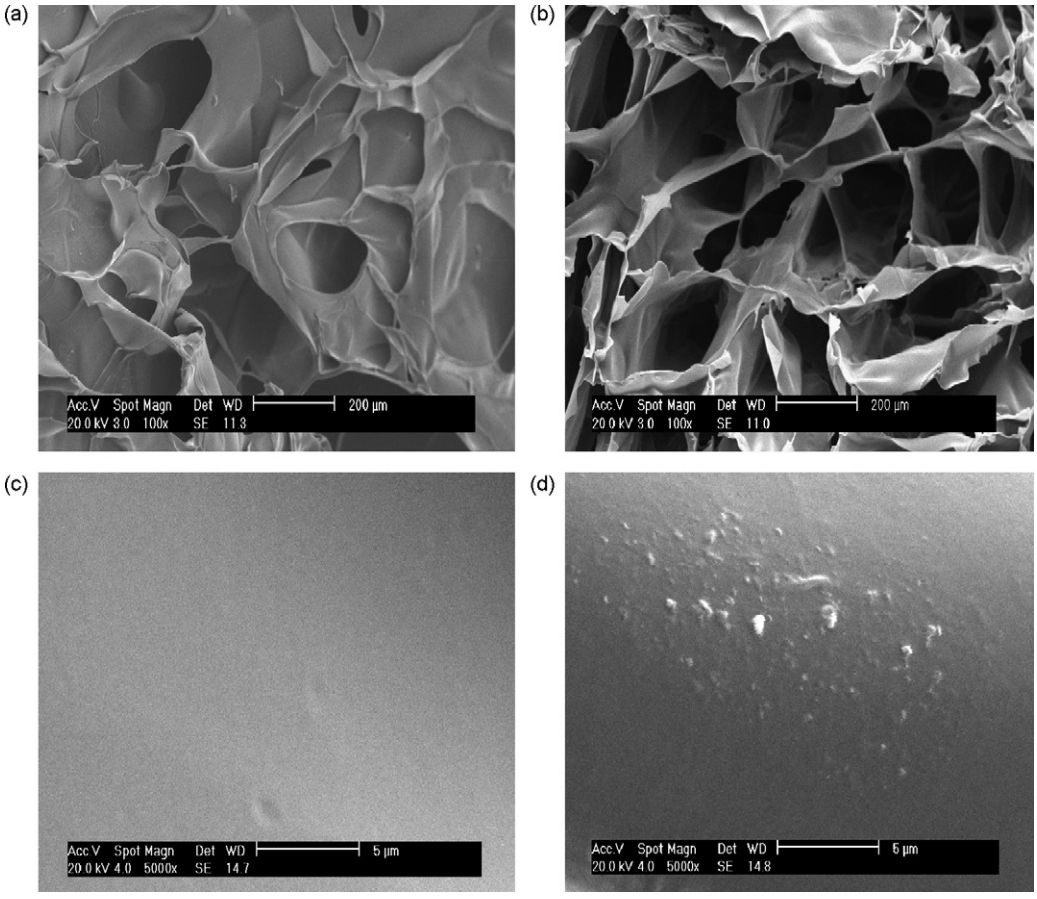

Fig. 5. SEM photographs of porous scaffolds: (a) cross-section of Cs–Gel–Ted scaffold; (b) cross-section of Cs–Gel–PLAE–Ted scaffold; (c) wall surface of pores of Cs–Gel–Ted scaffold; (d) wall surface of pores of Cs–Gel–PLAE–Ted scaffold.

3.4. Drug release behaviors

3.4.1. Drug loading performance

Apply the following equations to calculate drug encapsulation efficiency (EE) and drug loading content (LC) of PLAE–Ted nanospheres.

$$\mathrm{EE} = \frac{\text{total amount of drug} - \text{amount of free drug}}{\text{total amount of drug}} \times 100\%$$

$$\mathrm{LC} = \frac{\text{total amount of drug} - \text{amount of free drug}}{\text{polymer weight}} \times 100\%$$

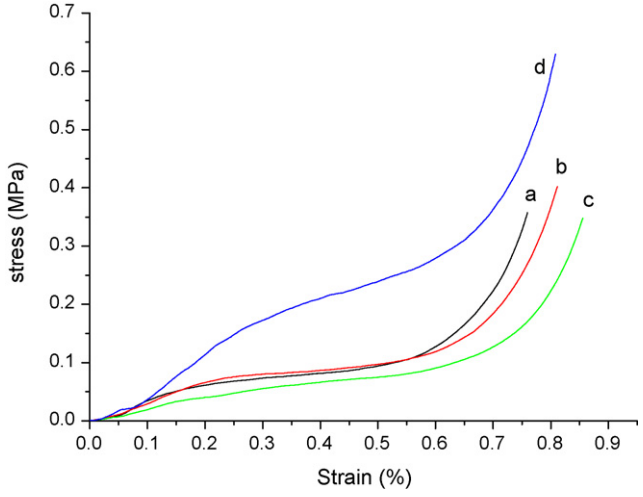

Fig. 6. Compressive stress–strain curves of Cs–Gel and Cs–Gel–PLAE scaffolds: (a) Scaffold-5; (b) Scaffold-6; (c) Scaffold-8; (d) Scaffold-1.

Fig. 7 shows the effects of Ted addition amount on the entrapment efficiency and drug loading content of PLAE–Ted nanospheres. When the initial added amount of Ted versus the constant amount of PLAE was increased, both drug loading content and drug encapsulation efficiency were enhanced.

3.4.2. Drug release from PLAE–Ted nanospheres

The Ted release properties from PLAE–Ted nanospheres were investigated using a dialysis membrane in phosphate

Table 3
Compressive modulus of different scaffolds

Code	Compressive modulus (MPa)
Scaffold-1	0.3208 ± 0.0036
Scaffold-5	0.2261 ± 0.0032
Scaffold-6	0.2248 ± 0.0028
Scaffold-7	0.2194 ± 0.0026
Scaffold-8	0.1743 ± 0.0024

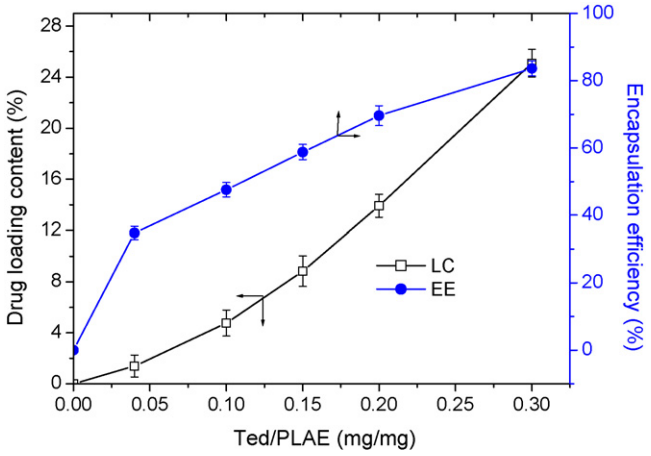

Fig. 7. Effects of the initial Ted addition amount on the Ted entrapment efficiency and Ted loading content of PLAE–Ted nanospheres.

buffered solution (pH 7.4, 37 °C). Plots of accumulative release percentage of Ted based on loading amount versus time are shown in Fig. 8. Since the initial burst effect was not observed in all examined samples, it confirmed that PLAE–Ted nanospheres could be prepared without any residual drug on their surfaces. While the free Ted exhibited rapid release behavior of above 90% within about 15 h, the Ted loaded into the inner core of nanospheres showed significant sustained release characteristics of less than 50% after 7 days.

The effect of drug loading amount on the drug release rate from the microspheres may vary when different drugs or polymers are used. Even in the case of the same system, i.e. paclitaxel-encapsulated PLA of PLGA microspheres, the results reported from different laboratories could be different (Liggins et al., 2000; Ruan and Feng, 2003). Fig. 8 shows the in vitro release curves of our PLAE–Ted nanospheres which had different Ted loading amounts. It can be seen that higher loading resulted in slower release. Because the drug dissolution in PLAE nanospheres and the degradation rate of PLAE should be similar for these samples, the effect of drug loading amount on the in

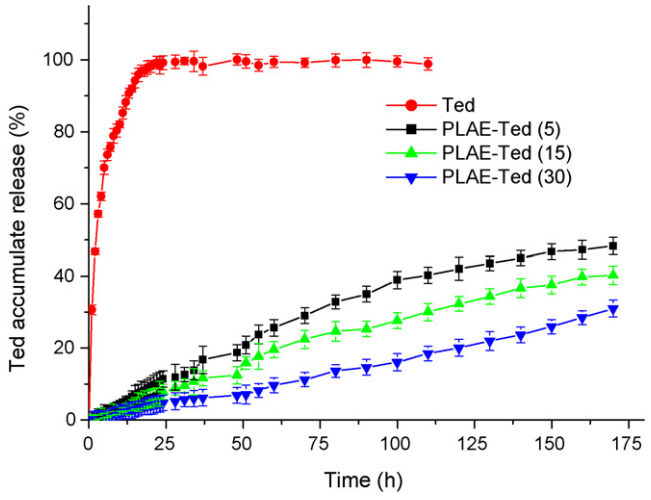

Fig. 8. Release profiles of Ted from PLAE–Ted nanospheres in pH 7.4 PBS at 37 °C.

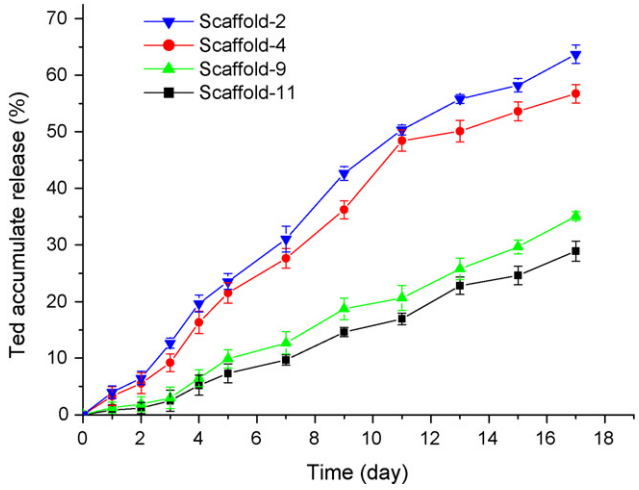

Fig. 9. Release curves of Ted from Cs–Gel–Ted and Cs–Gel–PLAE–Ted scaffolds.

vitro release profile could be attributed to the change of drug diffusivity caused by the different drug loading levels. Different amount of Ted might affect the microstructure in the PLAE–Ted nanospheres, which can be thus lead to different drug diffusion rate.

3.4.3. Drug release from porous scaffolds

In order to control the release behavior of Ted from scaffolds, here a Ted-loaded nanosphere embedded in chitosan–gelatin scaffolds was constituted (cf. Table 2, Scaffold-9 and Scaffold-11). As a control, Ted was also added into the scaffolds through mixing Ted with chitosan and gelatin solution directly in Cs–Gel–Ted scaffolds (cf. Table 2, Scaffold-2 and Scaffold-4). Fig. 9 displays release patterns of Ted from different kinds of 3D porous scaffolds. It can be seen that the release rate of Ted from Cs–Gel–PLAE–Ted scaffolds is slower and smoother than Cs–Gel–Ted scaffolds. Ted release rate from the scaffolds decreases when the drug loading content increases. The initial burst release was not observed in both kinds of the scaffolds. It is generally assumed that a drug is released by several mechanics: (a) Fickian diffusion through the polymer matrix, (b) diffusion through pores in the matrix, and (c) drug liberation by polymer erosion. For Cs–Gel–Ted or Cs–Gel–PLAE–Ted scaffolds, Ted is physically entrapped in Cs–Gel matrix. And in our case, degradation of chitosan or gelatin during the experiment period is ignorable. Therefore, it is speculated that the drug release from the scaffolds is carried out mainly through Fickian diffusion. For Cs–Gel–Ted scaffold, the main diffusion barrier is chitosan–gelatin network. Due to multiple delivering processes including barrier from both PLAE and Cs–Gel matrix, the drug release from Cs–Gel–PLAE–Ted scaffold became even more slowly than that of Cs–Gel–Ted scaffold. On the other hand, Ted is a kind of bisbenzylisoquinoline alkaloids; its methoxy groups could form hydrogen bond with hydroxyl, amine and carboxyl groups of chitosan and gelatin. This hydrogen bond interaction between Ted and the scaffolds limits the diffusion of Ted from the scaffolds to the release medium. So the burst release of Ted from Cs–Gel–Ted scaffolds does not appear. Although the com-

plex network of chitosan and gelatin can swell in water solution and that should be a positive factor to improve the Ted's diffusion, but Ted is a lipophilic alkaloid and its solubility in water is very low. That will result in the decrease of Ted's release rate from both kinds of the scaffolds along with the increase of drug loading amount in the scaffolds.

4. Conclusion

PLA/MePEG (PLAE) diblock copolymer was synthesized and its nanospheres were prepared by self-emulsification and solvent evaporating. The mean size of core/shell type PLAE nanosphere is about 60 nm. Lipophilic drug, e.g. tetrandrine was entrapped in nanovehicles with a relative high drug-loaded efficiency. The Ted-loaded nanospheres could be well embedded within Cs–Gel porous scaffold. The release rate of Ted from Cs–Gel–PLAE–Ted scaffolds is slower and smoother than that of Cs–Gel–Ted scaffolds. Ted release rate of Ted from Cs–Gel–Ted or Cs–Gel–PLAE–Ted scaffolds decreases along with the drug loading content increases. The initial burst release was not observed in both kinds of the scaffolds. That provides a room for sustained release lipophilic bioactive component from porous scaffold. That will offer a considerable potential in tissue engineering. The effects of the release behavior of Ted from Cs–Gel–PLAE–Ted scaffolds on different kinds of cells will be published in other place.

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