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A novel injectable local hydrophobic drug delivery system: Biodegradable nanoparticles in thermo-sensitive hydrogel☆

MaLing Gou[a], XingYi Li[a,1], Mei Dai[a,1], ChangYang Gong[a], XianHuo Wang[a], Yao Xie[b], HongXin Deng[a], LiJuan Chen[a], Xia Zhao[b], ZhiYong Qian[a,*], YuQuan Wei[a]

[a] State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China
[b] Department of Gynecology and Obstetrics, Second West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, China

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ABSTRACT

In this article, a novel local hydrophobic drug delivery system: nanoparticles in thermo-sensitive hydrogel, was demonstrated. First, honokiol, as a model hydrophobic drug, loaded poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) nanoparticles were prepared by emulsion solvent evaporation method, and then were incorporated into thermo-sensitive F127 hydrous matrix. The obtained injectable hydrophobic drug delivery system can act as a depot for sustained release of honokiol *in situ*. The lower critical solution temperature (LCST) of the composite matrix increases with increase in the mass of incorporated nanoparticles, or with decrease in the amount of residual organic solvent in the system. Honokiol release profile *in vitro* was studied, and the results showed that honokiol could be sustained released from the system. The described injectable drug delivery system might have great potential application for local delivery of hydrophobic drugs such as honokiol.

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1. Introduction

As advanced smart drug delivery systems, thermo-sensitive hydrogels play a very important role (Langer and Tirrell, 2004; Jeong et al., 1997; Kost and Langer, 2001; Schmaljohann, 2006). Pluronic F127 is a commercial thermo-sensitive hydrogel, which has potential application in experimental medicine (Moghimi and Hunter, 2000; Liu et al., 2007a; Dumortier et al., 2006). It exhibits reversible thermal gelation in aqueous solution at concentrations >20% (w/v). Aqueous F127 solution is liquid as sol phase at low temperature, but it rapidly gels at ~25 °C. After subcutaneous injection, the cold sol phase containing drugs can form a gel and acts as a depot for sustained release of drugs *in situ*. Administration of drug *in situ* assisted by injectable thermo-sensitive hydrogel, such as Pluronic F127, is an interesting route especially in cancer therapy (Tyagi et al., 2004; Prabaharan and Mano, 2006; Hatefi and Amsden, 2002). At the same time, Pluronic copolymers also have great potential application in overcoming drug resistance in cancer therapy (Kabanov et al., 2002). But when many hydrophobic drugs are considered to be locally delivered by thermo-sensitive hydrogels, its poor solubility make it not be well dispersed in the hydrous sol which greatly restricts the application of thermo-sensitive hydrogel in hydrophobic drug delivery. So, it is interesting for us to overcome the water-soluble problem of hydrophobic drug.

Application of nanotechnology for diagnosis, monitoring, disease therapy, and control of biological systems was referred to as "nanomedicine", and it received extensive attentions recently (Moghimi et al., 2005). Biodegradable polymeric nanoparticles were widely studied as drug delivery system (Allen and Cullis, 2004a; Farokhzad et al., 2006; Chawla and Amiji, 2002). It is a useful method to encapsulate drug in polymer nanoparticles to obtain well-dispersed drug slurry to overcome its poor solubility in aqueous solution (Tufts Center, in press; Sieverb et al., 2005). At the same time, developing nanomedicine also might provide a novel path to improve the uptake efficiency *in vivo*. Based on the above considerations, the idea to create a novel nanoparticles and thermo-sensitive hydrogel composite drug delivery system for administration of hydrophobic drugs *in situ* was generated. Meanwhile, this idea is encouraged by previous reports about micro- or

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* Corresponding author. Tel.: +86 28 85164063, fax: +86 28 85164060.
E-mail address: anderson-qian@163.com (Z. Qian).

[1] These authors are co-first authors for this paper, who did the even work with Gou ML.

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nano-particles in thermo-sensitive hydrogel composite drug delivery systems (Zhang et al., 2005; Barichello et al., 1999).

Previously, we have synthesized biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) triblock copolymer successfully (Gou et al., 2008; Huang et al., in press). Meanwhile, honokiol as a multi-function drug (Maruyama and Kuribara, 2000; Battle et al., 2005; Ahn et al., 2006) had been extracted (Chen et al., 2007) and its potential application in cancer therapy is being studied in our laboratory. Here, honokiol was used as a model hydrophobic drug to be entrapped into the PCEC nanoparticles to form stable slurry. Then, the honokiol loaded nanoparticles slurry was entrapped into commercial Pluronic F127 hydrous matrix as a model thermo-sensitive hydrogel. The described method and system might have great potential application in local drug delivery for hydrophobic drug such as honokiol.

2. Experiment part

2.1. Materials

Poly(ethylene glycol) (PEG, M_n = 4000), ε-caprolactone (ε-CL), stannous octoate ($Sn(Oct)_2$) and Pluronic F127 were purchased from Sigma (USA). Ethyl acetate (Et Ac) and dichloromethane (DCM) were purchased from KeLong Chemicals (Chengdu, China). Acetonitrile (AN) was purchased from Fisher Scientific (UK). Honokiol was purified in our laboratory by HSCCC method (Chen et al., 2007).

All the chemicals used in this work were all analytical pure grade, and used as received except PEG.

2.2. Synthesis of PCEC copolymer

PCEC copolymer was prepared by ring-opening polymerization method as previous description (Huang et al., in press; Zhou et al., 2003a). Briefly, calculated amount of ε-caprolactone and PEG4000 (with mass ratio = 24:1) were introduced into a dry glass ampoule under nitrogen atmosphere, and several drops of $Sn(Oct)_2$ was added as catalyst. During polymerization, the system was stirred slowly and the reaction temperature was kept at 130 °C. Six hours later, the system was rapidly heated to 180 °C under vacuum for another 20 min. After cooled to room temperature under nitrogen atmosphere, the just-obtained PCEC copolymer was first dissolved in methylene chloride and reprecipitated from the filtrate using excess AR grade cold petroleum ether. Then the mixture was filtered and vacuum dried to constant weight. The obtained purified PCEC copolymer was kept in air-tight bags in desiccators before use.

2.3. Preparation of honokiol loaded PCEC nanoparticles

Honokiol loaded or blank PCEC nanoparticles were prepared by emulsion solvent evaporation method. Briefly, 2 mL PCEC solution (60 mg/mL) in Et Ac containing honokiol (60 mg) or without honokiol was induced into 4 mL F127 aqueous solution under stirring at the speed of about 10,000 rpm by homogenizer (T10, IKA, Germany). After stable oil in water (O/W) emulsion formed, Et Ac was evaporated in rotator evaporator (BÜCHI, Switzerland) and nanoparticles were obtained. At last, the resultant nanoparticles slurry was adjusted to 4 mL by addition of distilled water.

2.4. Determination of drug loading (DL) and entrapment efficiency (EE)

First, 0.2 mL of drug loaded PCEC nanoparticles slurry was induced into pre-weighed EP tube and centrifuged at 13,000 rpm for 60 min. Then, the supernatant was fully removed and the remained deposit was lyophilized to constant weigh. Afterwards, the dried deposit was dissolved in 0.1 mL DCM and was diluted by acetonitrile. Meanwhile, the amount of honokiol in the solution was determined by HPLC. At last, drug loading (DL) and entrapment efficiency (EE) of drug-loaded nanoparticles were calculated according to Eqs. (1) and (2) (Jia et al., 2007):

$$\mathrm{DL}(\%)\frac{\text{amount of drug}}{\text{amount of polymer} + \text{drug}} \times 100 \quad (1)$$

$$\mathrm{EE}(\%) = \frac{\text{experimental drug loading}}{\text{nominal drug loading}} \times 100 \quad (2)$$

2.5. Incorporating nanoparticles in F127 sol

Calculated amount of F127 was well dissolved in prepared drug loaded or blank nanoparticles slurry at 4 °C to form solution (sol), and the total concentration of F127 was maintained at 20% (w/w).

2.6. Determination of the sol–gel transition temperature

In this paper, test-tube method was used to determine the sol–gel transition behavior (Gong et al., 2007). One milliliter of F127 sol containing blank PCEC nanoparticles in test tube was put in water-bath with temperature increased from 4 to 85 °C at the speed of ca. 1 °C/min, and the sol–gel transition temperature was recorded. The phase is regarded as "sol" when the matrix could flow, and the phase is regard as "gel" while it could not flow down when test tube inversed.

2.7. In vitro release of honokiol

According to JM Barichello's method (Barichello et al., 1999), a membraneless model was used to assay the release behavior of honokiol *in vitro*. In detail, 1 mL of prepared honokiol loaded PCEC nanoparticles slurry or F127 gel containing honokiol loaded PCEC nanoparticles was placed at the bottom of EP tube (15 mL) and 10 mL PBS (pH 7.4) containing Tween80 (0.5%) was added. The release study was performed in shaking air bath at 100 rpm, 37 °C. At predetermined time, the release medium was centrifuged at the speed of 14,000 rph for 60 min. Then, the deposit was re-dispersed in 10 mL of fresh PBS and re-added into the EP tube. The concentration of honokiol in the supernatant was determined by high-performance liquid chromatography (HPLC) and the cumulative release profile of honokiol was obtained.

2.8. High-performance liquid chromatography (HPLC)

The concentration of honokiol was determined by HPLC Instrument (Waters Alliance 2695, USA), and the samples were diluted by acetonitrile/water solution (1/1, v/v) before measurement. Solvent delivery system equipped with a column heater and a plus autosampler. Detection was taken on a Waters 2996 detector. Chromatographic separations were performed on a reversed phase C_{18} column (4.6 mm × 150 mm-5 μm, Sunfire Analysis column). And the column temperature was kept at 28 °C. Acetonitrile/water (60/40, v/v) was used as eluent at a flow rate of 1 mL/min. The standard curve equation is: $H = 105{,}000X + 4680$ (H: the area of peak; X: the concentration of honokiol) and the correlation coefficient is 0.999994.

2.9. Laser diffraction particle size analyser

The particle size and distribution were determined by Malvern Nano-ZS 90 laser particle size analyzer (Malvern, Nano ZS, UK). The measurement temperature was kept at 25 °C.

2.10. Transmission electron microscope (TEM)

The morphology of prepared honokiol nanoparticles was observed under a transmission electron microscope (TEM) (H-6009IV, Hitachi, Japan): the nanoparticles were diluted with distilled water and placed on a copper grid covered with nitrocellulose. The sample was negatively stained with phosphotungstic acid and dried at room temperature.

2.11. Gas chromatography-flame ionization detection system

The determination of ethyl acetate (Et Ac) was performed using an Agilent 6890N gas chromatograph (Agilent Technologies, USA) equipped with a FID detector and with a Alltech Ec-Wax column (30 m × 0.53 mm i.d. and 0.25 mm film thickness). Splitless injection method was used with a deactivated, splitless inlet liner with adsorbent material and taper (Agilent Technologies). The injector and detector temperatures were both 250 °C. The carrier gas was nitrogen (≥ 99.99%) with a flow rate of 3 mL/min. The hydrogen and air-flow rates of FID were adjusted at 35 and 350 mL/min, respectively. Oven temperature was set at 80 °C held for 10 min. One milliliter of sample was injected into system and the retention time of Et Ac was 3.543 min.

3. Results

The process scheme of the demonstrated nanoparticles in thermo-sensitive hydrogel composite drug delivery system was presented in Fig. 1. First, honokiol was loaded in biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) nanoparticles by emulsion solvent evaporation method, and honokiol loaded PCEC nanoparticles slurry was obtained. Then, Pluronic F127 was added to the drug loaded nanoparticles slurry to form solution (sol) at 4 °C for further application. After the prepared formulation was injected into body, the complex F127 gel forms, and it can act as a depot for the sustained release of drugs. The morphology *in vitro* of the demonstrated drug delivery system

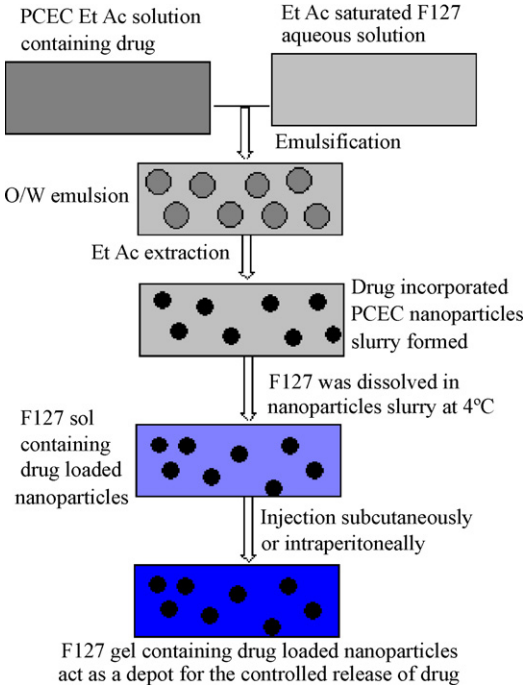

Fig. 1. The process scheme of the demonstrated injectable local drug delivery system: nanoparticles in thermo-sensitive hydrogel.

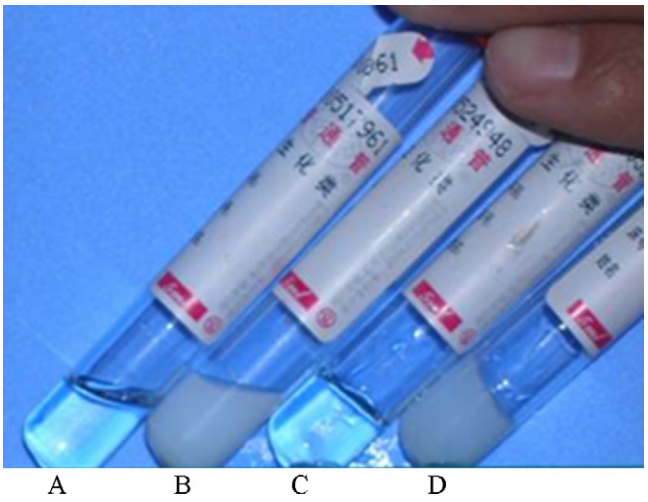

Fig. 2. Directly observation of obtained drug delivery system: nanoparticles in thermo-sensitive hydrogel *in vitro*. After the tube was taken out of the water bath, the photo was taken immediately. (A) F127 sol, at the concentration of 20% (w/w), at 4 °C; (B) F127 sol at the concentration of 20% (w/w) containing honokiol loaded nanoparticles slurry at 4 °C; (C) F127 gel, at the concentration of 20% (w/w), at 37 °C; (D) F127 gel, at the concentration of 20% (w/w), containing honokiol loaded nanoparticles slurry at 37 °C.

was shown in Fig. 2. At 4 °C, the sol could flow because of its low viscosity. When the temperature increased to 37 °C, gel formed and it could not flow again. Meanwhile, the morphology *in vivo* of the described delivery system could be seen in Fig. 3. It could be directly observed that the demonstrated drug delivery system acted as a depot *in situ* after S.C. injection and it gradually was removed with lapse of time as shown in Fig. 3 (from Fig. 3(a)–(d)). In Fig. 3(d), it could be found that the depot almost could not be obviously seen in the photo even 6 h later after injection. But the depot was still under the skin and the exact fact was that, due to the low strength of the formed hydrogel, the depot was pressed into a lamella under skin because of the mouse's movement.

3.1. Characterization of drug loaded PCEC nanoparticles

Previously, biodegradable PCEC triblock copolymer based on ε-caprolactone and PEG was synthesized by ring-opening polymerization, and the macromolecular weight (Mw) was 3.6×10^4 detected by gel permeation chromatography (GPC) (Huang et al., in press). The mean particle size of the prepared blank and honokiol loaded PCEC nanoparticles in this work was 148 and 157 nm respectively, and the particle size distribution spectra were shown in Fig. 4. The size distribution spectra indicated that mono-dispersed PCEC nanoparticles were obtained, and particle size increased very slightly for the incorporated honokiol. At the same time, the morphology of honokiol loaded PCEC nanoparticles determined by TEM was shown in Fig. 5 and the nearly spherical nanoparticles could be observed. Loading efficiency and drug loading of the prepared honokiol loaded PCEC nanoparticles were about 98 and 32.9%, respectively. Otherwise, it is interesting that the honokiol loaded nanoparticles could be released from F127 gel, and the mean particle size of the released nanoparticles was 160 nm which implied that the honokiol could be released from the gel at the form of honokiol loaded nanoparticles.

3.2. Effect of the content of incorporated nanoparticles and residual organic solvent on the sol–gel transition temperature

When nanoparticles were incorporated into F127 hydrous matrix, effect of the content of nanoparticles on the sol–gel

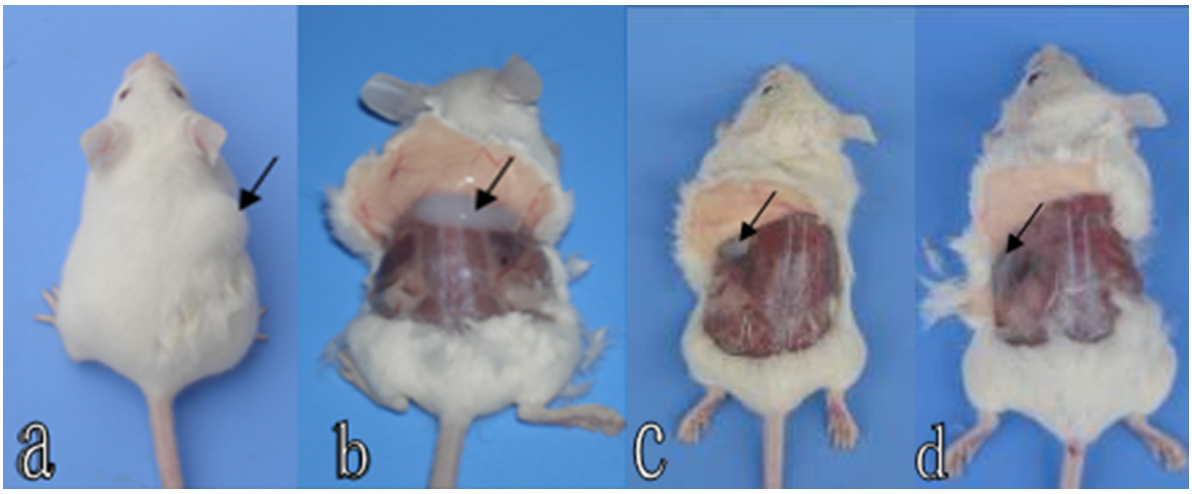

Fig. 3. The morphology of subcutaneously injected F127 gel containing honokiol loaded PCEC nanoparticles *in vivo*. (a) The mouse after injection of 0.2 mL of the F127 sol (20%, w/w) containing 10 mg of honokiol loaded PCEC nanoparticles; (b) the honokiol loaded nanoparticles incorporated F127 gel depot under skin 5 min later after injection; (c) the drug loaded depot under skin 4 h later after injection; and d) the depot under skin 6 h later after injection.

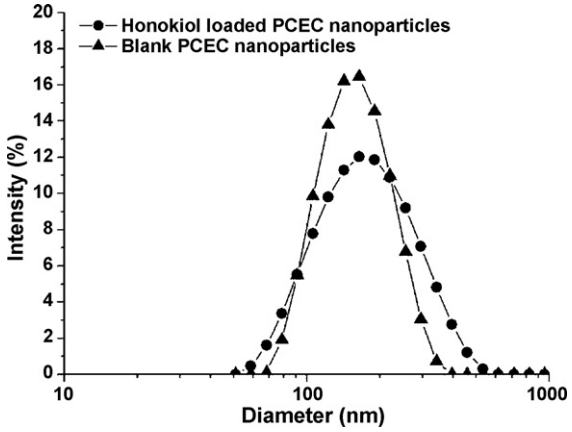

Fig. 4. Particle size distribution spectra of prepared blank PCEC and honokiol loaded PCEC nanoparticles.

transition temperature was investigated and the results were presented in Fig. 6. It could be found that the LCST increased and the gel zone became narrower with increase in the amount of nanoparticles. Otherwise, in this delivery system, residual Et Ac in the nanoparticles slurry could not be avoided, but actually the residual Et Ac content was 87.4 μg/mL determined by GC, which is lower than 0.01% in the system. Here, the effect of Et Ac on the sol–gel transition temperature of the system was studied and the result was shown in Fig. 7. It indicated that the LCST decreased with increase in the content of Et Ac.

3.3. *In vitro release profile*

The release profiles of honokiol were studied *in vitro*, and the results were shown in Fig. 8 which indicated that honokiol could be sustained released from PCEC nanoparticles and nanoparticles-F127 hydrogel matrix. When honokiol released from the nanoparticles, visible burst release occurred followed by slowly release ratio. At the same time, it could be observed that burst release was weakened when honokiol released from the composite system.

4. Discussion

Local delivery is an important method for both local therapy and systematic therapy. In this article, a novel composite local delivery system assisted by F127 for honokiol was demonstrated. About F127 hydrous matrix, as the temperature increases, equilibrium shifts from unimers to spherical micelles, which resulted in an increase in micelle volume fraction (Φ_m). When Φ_m is >0.53, the system becomes a gel by micelle packing (hard-sphere crystallization). The transition mechanism from sol-to-gel is related to the shrinkage of poly(ethylene glycol) (PEO) corona of the micelles because of temperature effects on PEO solubility and interaction of PEO chains with the poly(propylene glycol) (PPO) hard core (Jeong and Gutowska, 2002).

As shown in Fig. 6, the LCST increased and the gel zone became narrower with increase in the mass of nanoparticles which might be due to two reasons as follow. First, it might be due to physical interaction between F127 molecules and PCEC nanoparticles, just like PCEC nanoparticles played a role as cross-linker which is con-

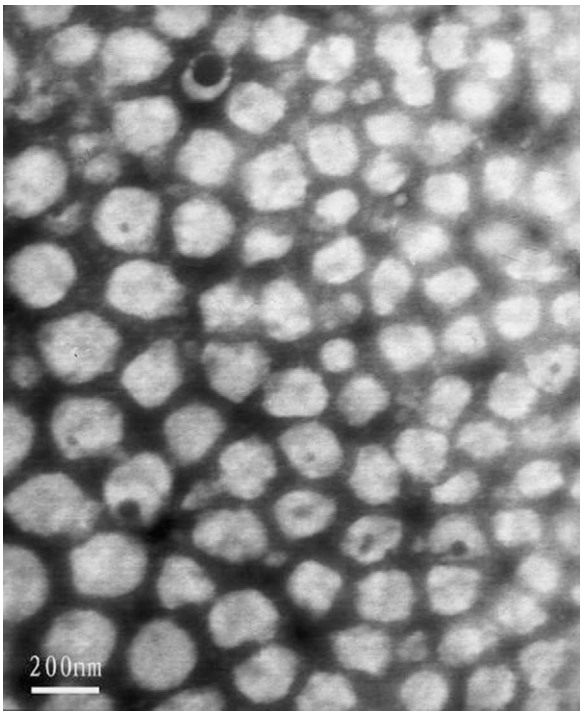

Fig. 5. The TEM image of prepared honokiol loaded PCEC nanoparticles.

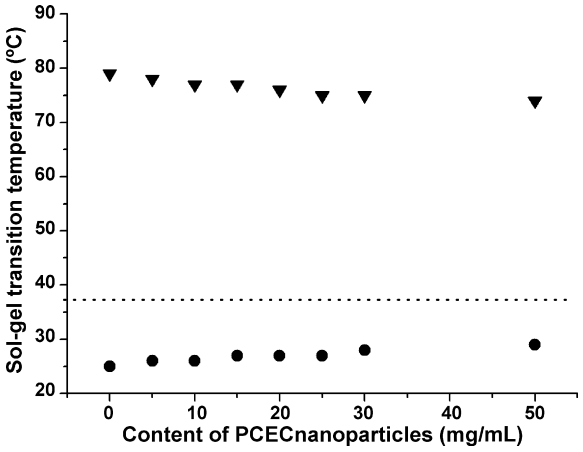

Fig. 6. Effect of content of nanoparticles incorporated in F127 hydrous matrix on the sol–gel transition temperature. (●) F127 sol transits to gel when temperature increases from 4 to 37 °C at the speed of 1 °C/min; (▼) F127 gel transits to sol when temperature continues to increase after gel forms at the speed of 1 °C/min; (. . .) 37 °C, the body temperature.

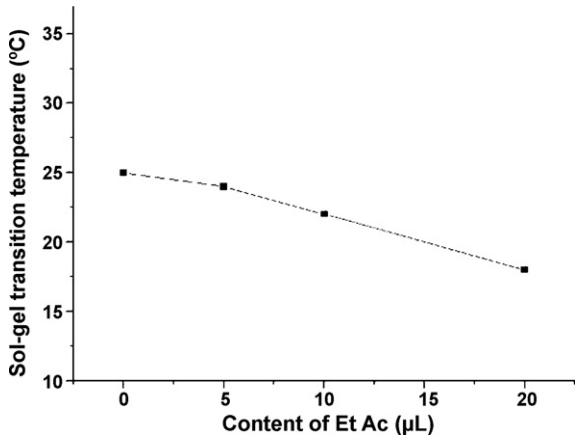

Fig. 7. Effect of residual Et Ac content on the LCST of F127 matrix. Different volume of Et Ac was dissolved in 0.5 mL of F127 sol (20%, w/w) at 4 °C. F127 sol transit to gel while temperature increases from 4 to 37 °C at the speed of 1 °C/min.

sistent with those behaviors when magnetite nanoparticles was incorporated into F127 matrix and clay particles were dispersed into PNIPAAm system (Liu et al., 2007b; Haraguchi et al., 2002). Secondly, the prepared honokiol loaded PCEC nanoparticles might

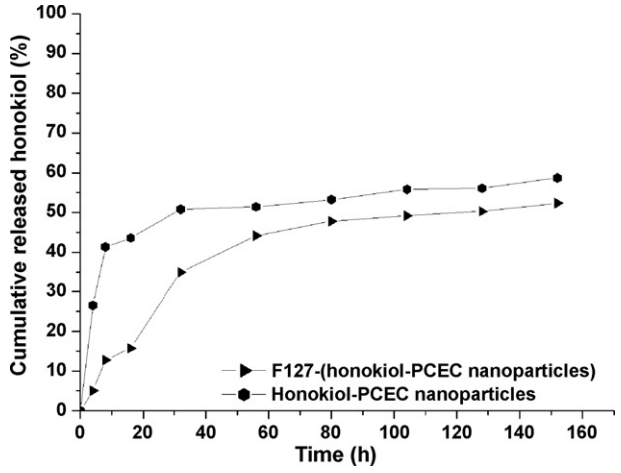

Fig. 8. *In vitro* release profile of honokiol from different matrix in PBS (pH 7.4) at 37 °C.

have core-shell structure and the nanoparticles were covered by hydrophilic chains such as PEG or PEO which might be disadvantage for F127 unimers to form spherical micelles to form gel which made the LCST higher and gel region narrower (Schmaljohann, 2005). Otherwise, it was suggested that the LCST decreased with increase of the amount of Et Ac, which might be due to that the residual Et Ac changed the hydrophilic/hydrophobic balance and made equilibrium shifted from unimers to spherical micelles more easily. In this paper, the residual Et Ac in the prepared composite delivery system was 87.4 μg/mL which was very low and could be tolerated by human beings.

Nanoparticles had been widely used in drug delivery (Wagner et al., 2006), and emulsion solvent evaporation method is one of the most important methods to prepare polymer nanoparticles (O'Donnell and McGinity, 1997). Traditionally, almost half of new molecular entities identified by pharmaceutical industry screening programs have failed to be developed because of poor water-solubility, which makes their formulation difficult or even impossible and one essential factor in the drug development equation is drug solubility (Tufts Center, in press). Nanotechnology provides an interesting method to overcome the poor water-solubility of hydrophobic drug (Allen and Cullis, 2004b).

In this paper, when nanoparticles were prepared, F127 was employed as stabilizer, which is approved by FDA due to its low toxicity. The nanoparticles slurry was directly used without centrifugation/wash to remove the free surfactant, which always induced aggregation of nanoparticles. At the same time, it is difficult to fully precipitate nanoparticles by centrifugation which results in wasting drug. At last, it was implied that honokiol could be released from the nanoparticles or nanoparticles-F127 composite matrix according to *in vitro* release results. It is known that PCL, as a kind of polyesters, is biodegradable based on hydrolization and enzymatic degradation (Sinha et al., 2004; Gan et al., 1999). At the same time, it also was shown by many studies that PCL–PEG–PCL, a triblock copolymer based on PCL and PEG, could be degraded *in vitro* and *vivo* due to the hydrolization and enzymatic degradation (He et al., 2003; Zhou et al., 2003b). Otherwise, degradation of biodegradable nanoparticles always can be divided into two mechanisms: surface erosion and bulk eroding. And at most time, degradation of biodegradable micro-/nano-particles is due to the combination effects of surface erosion and bulk eroding (Langer, 2003). About drug-loaded nanoparticles, drug can be released from nanoparticles due to diffusion and degradation of nanoparticles (Xiong et al., 2005). In this paper, on the first day, honokiol was burst released on the first day. In this stage, diffusion mechanism might take the major role. Afterwards, the release speed of honokiol was slowed which might be due that degradation of nanoparticles, instead of diffusion, take the major role in this stage. It was reported that the degradation of PCL–PEG–PCL nanoparticles also was due to the combination effects of surface erosion and bulk burst, and it could hold several months *in vivo*. So the release speed of honokiol after 80 h was very slow but release was still continued. For honokiol-loaded nanoparticles, drug could be released due to drug diffusion and degradation of polymer matrix. For this composite drug delivery system, three release processes might be included: drug released from the gel, nanoparticles released from the gel, and drug released from nanoparticles. Because drug loaded nanoparticles was delayed to be released from the gel, the drug release speed of composite system was slowed down, and the burst release was reduced compared to drug loaded nanoparticles.

In short, for the composite drug delivery system described in this article, the water-soluble problem of honokiol was overcame by nanotechnology; the local delivery was achieved associated by thermo-sensitive hydrogel; and drug could be released slowly from the system. At last, the PCEC and F127 are both low toxic which also

provide the potential application as local hydrophobic drug delivery system in clinical.

5. Conclusion

In this paper, we have successfully prepared a novel injectable hydrophobic drug delivery system: nanoparticles in thermosensitive hydrogel. It has these properties: (1) local delivery of hydrophobic drug; (2) sustained release; (3) less drugs to be used; (4) biocompatible and injectable. The obtained injectable drug delivery system might have great potential application for local administration of poorly soluble drugs such as honokiol.

References

Ahn, K.S., Sethi, G., Shishodia, S., Sung, B., Arbiser, J.L., Bharat, B., Aggarwal, 2006. Honokiol potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through modulation of nuclear factor-κB activation pathway. Mol. Cancer Res. 4, 621–633.

Allen, T.M., Cullis, P.R., 2004a. Drug delivery systems: entering the mainstream. Science 303, 1818–1822.

Allen, T.M., Cullis, P., 2004b. Drug delivery systems: entering the mainstream. Science 303, 1818–1822.

Barichello, J.M., Morishita, M., Takayama, K., Nagai, T., 1999. Absorption of insulin from pluronic F-127 gels following subcutaneous administration in rats. Int. J. Pharm. 184, 189–198.

Battle, T.E., Arbiser, J., Frank, D.A., 2005. The natural product honokiol induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells. Blood 106, 690–697.

Chawla, J.S., Amiji, M.M., 2002. Biodegradable poly(ε-caprolactone) nanoparticles for tumor targeted delivery of tamoxifen. Int. J. Pharm. 249, 127–138.

Chen, L., Zhang, Q., Yang, G., Fan, L., Tang, J., Garrard, I., Ignatova, S., Fisher, D., Sutherland, I.A., 2007. Rapid purification and scale-up of honokiol and magnolol using high-capacity high-speed counter-current chromatography. J. Chromatogr. A 1142, 115–122.

Dumortier, G., Grossiord, J.L., Agnely, F., Chaumeil, J.C., 2006. A review of Poloxamer 407 pharmaceutical and pharmacological characteristics. Pharm. Res. 23, 2709–2728.

Farokhzad, O.C., Cheng, J., Teply, B.A., Sherifi, I., Jon, S., Kantoff, P.W., Richie, J.P., Langer, R., 2006. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. PNAS 103, 6315–6320.

Gan, Z.H., Fung, J.T., Jing, X.B., Wu, C., Kuliche, W.K., 1999. A novel laser light-scattering study of enzymatic biodegradation of poly(ε-caprolactone) nanoparticles. Polymer 40, 1961–1967.

Gong, C.Y., Qian, Z.Y., Liu, C.B., Huang, M.J., Gu, Y.C., Wen, Y.J., Kan, B., Wang, K., Dai, M., Li, X.Y., Gou, M.L., Tu, M.J., Wei, Y.Q., 2007. A thermosensitive hydrogel based on biodegradable amphiphilic poly(ethylene glycol)-polycaprolactone-poly(ethylene glycol) block copolymers. Smart Mater. Struct. 16, 927–933.

Gou, M.L., Wang, H., Huang, M.J., Tang, Y.B, Qian, Z.Y., Wen, Y.J., Huang, A.L., Lei, K., Gong, C.Y., Li, J., Jia, W.J., Liu, C.B., Deng, H.X., Tu, M.J., Chao, G.T., 2008 Preparation and characterization of magnetic poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) microspheres. J. Mater. Sci.: Mater. Med. 19, 1033–1041.

Haraguchi, K., Takehisa, T., Fan, S., 2002. Effects of clay content on the properties of nanocomposite hydrogels composed of poly (*N*-isopropylacrlamide) and clay. Macromolecules 35, 10162–10171.

Hatefi, A., Amsden, B., 2002. Biodegradable injectable in situ forming drug delivery systems. J. Control. Release 80, 9–28.

He, F., Lia, S., Vert, M., Zhuo, R., 2003. Enzyme-catalyzed polymerization and degradation of copolymers prepared from ε-caprolactone and poly(ethylene glycol). Polymer 44, 5145–5151.

Huang, M.J., Gou, M.L., Huang, A.L., Kan, B., Yang, J.L., Tu, M.J., Wei, Y.Q., Qian, Z.Y., in press. One-step preparation of cationic PCL–PEG–PCL (PCEC) nanoparticles for plasmid DNA delivery. J. Biomed. Mater. Res. A. doi:10.1002/jbm.a.31704.

Jeong, B., Gutowska, A., 2002. Lessons from nature: stimuli-responsive polymers and their biomedical applications. Trends Biotechnol. 20, 305–311.

Jeong, B., Bae, Y.H., Lee, D.S., Kim, S.W., 1997. Biodegradable block copolymers as injectable drug-delivery systems. Nature 388, 860–862.

Jia, W.J., Liu, J.G., Zhang, Y.D., Wang, J.W., Wang, J., et al., 2007. Preparation, characterization, and optimization of pancreas-targeted 5-Fu loaded magnetic bovine serum albumin microspheres. J. Drug Target 15, 140–155.

Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., 2002. Pluronic block copolymers for overcoming drug resistance in cancer. Adv. Drug Deliv. Rev. 54, 759–779.

Kost, J., Langer, R., 2001. Responsive polymeric delivery systems. Adv. Drug Deliv. Rev. 46, 125–148.

Langer, R., 2003. Advances in biomaterials, drug delivery, and bionanotechnology. AICHE J. 49, 2990–3006.

Langer, R., Tirrell, D.A., 2004. Designing materials for biology and medicine. Nature 428, 487–492.

Liu, Y., Lu, W.L., Wang, J.C., Zhang, X., Zhang, H., Wang, X.Q., Zhou, T.Y., Zhang, Q., 2007a. Controlled delivery of recombinant hirudin based on thermo-sensitive Pluronic® F127 hydrogel for subcutaneous administration: In vitro and in vivo characterization. J. Control. Release 117, 387–395.

Liu, T.Y., Hu, S.H., Hu, S.H., Tsai, S.P., Chen, S.Y., 2007b. Preparation and characterization of thermal-sensitive ferrofluids for drug delivery application. J. Mag. Mag. Mater. 310, 2850–2852.

Maruyama, Y., Kuribara, Hi, 2000. Overview of the pharmacological features of honokiol. CNS Drug Rev 6, 35–44.

Moghimi, S.M., Hunter, A.C., 2000. Poloxamers and poloxamines in nanoparticle engineering and experimental medicine. Trends Biotechnol. 18, 4120–4420.

Moghimi, S.M., Hunter, A.C., Murray, J.C., 2005. Nanomedicine: current status and future prospects. FASEB J. 19, 311–330.

O'Donnell, P.B., McGinity, J.W., 1997. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Deliv. Rev. 28, 25–42.

Prabaharan, M., Mano, J.F., 2006. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. Macromol. Biosci. 6, 991–1008.

Schmaljohann, D., 2005. Thermo-responsive polymers and hydrogels in tissue engineering. e-Polymers 2005, no. 021.

Schmaljohann, D., 2006. Thermo- and pH-responsive polymers in drug delivery. Adv. Drug Deliv. Rev. 58, 1655–1670.

Sieverb, R.S., Ogden, J., Billings, M.P., 2005. Nanotechnology approaches to solving the problems of poorly water-soluble drugs. Drug Discov. World, 71–76 (summer).

Sinha, V.R., Bansal, K., Kaushik, R., Kumria, R., Trehan, A., 2004. Poly-ε-caprolactone microspheres and nanospheres: an overview. Int. J. Pharm. 278, 1–23.

Tufts Center for the Study of Drug Development, Tufts University, Boston, MA, USA. http://csdd.tufts.edu.

Tyagi, P., Li, Z., Chancello, M., De Groat, W.C., Yoshimura, N., Huang, L., 2004. Sustained intravesical drug delivery using thermosensitive hydrogel. Pharm. Res. 21, 832–837.

Wagner, W., Dullaart, A., Bock, A.K., Zwech, A., 2006. The emerging nanomedicine landscape. Nat. Biotechnol. 24, 1211–1217.

Xiong, X.Y., Tam, K.C., Gan, L.H., 2005. Release kinetics of hydrophobic and hydrophilic model drugs from Pluronic F127/poly(lactic acid) nanoparticles. J. Control. Release 103, 73–82.

Zhang, X.Z., Lewis, P.J., Chu, C.C., 2005. Fabrication and characterization of a smart drug delivery system: microsphere in hydrogel. Biomaterials 26, 3299–3309.

Zhou, S., Deng, X., Yang, H., 2003a. Biodegradable copolymer of poly(ethylene glycol)-co-poly(ε-caprolactone): characterization and its use in controlled release system. Biomaterials 24, 3563–3570.

Zhou, S.B., Deng, X.M., Yang, H., 2003b. Biodegradable poly(ε-caprolactone)-poly(ethylene glycol) block copolymers: characterization and their use as drug carriers for a controlled delivery system. Biomaterials 24, 3563–3570.