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Self-assembled honokiol-loaded micelles based on poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) copolymer☆

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ABSTRACT

Self-assembled polymeric micelles are widely applied in drug delivery system (DDS). In this study, honokiol (HK) loaded micelles were prepared from biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) copolymers. Micelles were prepared by self-assembly of triblock copolymer PCEC in distilled water triggered by its amphiphilic character without any organic solvent. Drug loading and encapsulation efficiency were determined by adjusting the weight ratio of HK and PCEC. The particle size and zeta potential distribution of obtained micelles were determined using Malvern laser particle sizer, and spherical geometry were observed on atomic force microscope (AFM). Otherwise, the thermo-sensitivity of honokiol-loaded micelles was monitored. And the cytotoxicity results of drug loaded micelles showed that the encapsulated honokiol remained potent antitumor effect. Moreover, *in vitro* release profile demonstrated a significant difference between rapid release of free honokiol and much slower and sustained release of HK-loaded micelles. These results suggested that we have successfully prepared honokiol-loaded micelles in an improved method which is safer and more efficient. The prepared micelles might be potential carriers for honokiol delivery in cancer chemotherapy.

1. Introduction

Nanobiotechnologies are accelerating the identification and evaluation of drug candidates. In recent years, nanobiomaterials such as polymer-drug conjugates (Kopecek et al., 2001), liposomes (Takeuchi et al., 2004), nanoparticles (Vargas et al., 2004) and polymeric micelles (Ideta et al., 2005; Jang et al., 2006) have been considered as potential carriers for hydrophobic drug delivery. Micelle, with intrinsic core-shell architecture demonstrates a series of attractive properties in drug delivery systems for increasing drug stability, drug solubility, and passive target effects (Croy and Kwon, 2006). To date, an increasing number of studies have been focused on micelles with anticancer drugs core-encapsulated in. Several examples of micelles as ideal drug carriers with tissue penetrating ability and controlled drug release have been reported (Lin et al., 2006; Cai et al., 2007; Nakayama et al., 2007; Choi et al., 2006), in particular, two micellar formulations, a paclitaxel-incorporated micellar nanoparticle (PTX (NK105)) (Hamaguchi et al., 2007) and a polymeric micelle carrier system for doxorubicin (DOX (NK911)) (Nakanishi et al., 2001), are currently being studied in phase I and phase II clinical trials, respectively.

Micelles prepared from synthetic biodegradable block copolymers are widely applied in drug delivery system (DDS) due to their core-shell geometry. Amphiphilic block copolymers are comprised of hydrophobic (water insoluble) and hydrophilic (water soluble) parts. The hydrophobic segments become packed together in aggregates (core) which serves as a potent nanocontainer of hydrophobic compounds while hydrophilic domain (shell) serves as a stabilizing interface of particles. Many biodegradable amphiphilic block copolymers based on aliphatic polyesters, e.g. poly(lactide)-poly(ethylene glycol) copolymers (PLA-*b*-PEG), poly(lactide-glycolide)-poly(ethylene glycol) copolymers (PLGA-*b*-PEG), and poly(ε-caprolactone)-poly(ethyleneglycol) copolymer (PCL-*b*-PEG) have been synthesized and frequently applied in DDS due to their great biocompatibility (Zhou et al., 2003; Shuai et al., 2003; Peng et al., 2008a). Emulsion solvent evaporation method

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and dialysis have been widely used to prepare micelles (Gou et al., 2008a; Qiu and Bae, 2007). However, little work was done to prepare micelles without organic solvent just depending merely on the self-assembly of block polymers in distilled water. The self-assembling motif being exploited can be of tremendous benefit for the absence of organic solvent therefore lower toxicity and material cost in synthesis and micellization.

Previously, we have successfully synthesized biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) triblock copolymer (Gou et al., 2008b; Jia et al., 2008). Meanwhile, honokiol, a potent anti-cancer agent, had been extracted (Chen et al., 2007). Honokiol is an active component isolated and purified from Chinese traditional herb magnolia which was demonstrated to inhibit growth and induce apoptosis of different cancer cell lines (Liu et al., 2008; Dikalov et al., 2008; Li et al., 2008), but high hydrophobicity therefore restrained its further application in cancer chemotherapy. In this study, biodegradable honokiol-loaded polymeric micelles were prepared from self-assembly of triblock PCEC copolymer, and were characterized in detail. The prepared honokiol micelles might have great potential clinic application as a new dosage form.

2. Experimental

2.1. Materials

Poly(ethylene glycol) (PEG, $M_n = 4000$, Fluka, USA), ε-Caprolactone (ε-CL, Alfa Aesar, USA), Stannous octoate ($Sn(Oct)_2$, Sigma, USA), Dulbecco's modified Eagle's medium (DMEM, Sigma, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA), and dimethyl sulfoxide (DMSO) were used without any further purification. Honokiol were isolated and purified in our lab (Chen et al., 2007). All the materials used in this article were analytic reagent (AR) grade and used as received except honokiol.

2.2. Synthesis of PCL-PEG-PCL (PCEC) copolymer

PCL-PEG-PCL (PCEC) copolymer with the designed molecular weight of 6000 was synthesized by ring-opening polymerization of ε-CL and PEG ($M_w = 4000$) using $Sn(Oct)_2$ as catalyst according to our previous reports (Gou et al., 2008a). The obtained PCEC copolymer was purified and kept in air-tight bags before further application.

2.3. Preparation of blank or honokiol-loaded PCEC micelles

Ten milligrams of PCEC was dissolved in 1 mL of distilled water at 50 °C. Five minutes later, PCEC micelles formed due to thermal induced self-assembling. Then, the PCEC micelles were cooled to 37 °C for further application and characterization. Drugs loaded PCEC micelles were prepared by direct dissolution method assisted by ultrasonication. As model drug, calculated amount of honokiol was added into PCEC micelles solution at 37 °C under ultrasonication (JY92-2D, Ningbo Scientz Biotechnology Co., China). Thirty minutes later, the suspension were ultracentrifuged and filtered with a syringe filter (pore size: 220 nm) (Millex-LG, Millipore Co., USA) to remove the insoluble drugs. And the filtrate was used for further application or characterization.

2.4. Characterization of honokiol-loaded PCEC micelles

The particle size and zeta potential of prepared micelles was determined by Malvern Nano-ZS 90 laser particle size analyzer after equilibration for 10 min. And all results were the mean of three independent test runs.

The morphological characteristics of the nanoparticles were examined by Atomic Force microscope (AFM, SPA-400, Seiko Instruments Inc, Japan). The obtained micelles were diluted with distilled water and placed on a mica surface. The surface morphology of micelles was observed after dried at room temperature.

2.5. Crystallographic study

Crystallographic assay was performed on honokiol-loaded micelles powered by X-ray Diffractometer (X'Pert Pro, Philips, Netherlands) using Mo Kα radiation.

2.6. In vitro drug release behavior

In vitro release behavior of honokiol from drug loaded PCEC micelles was studied using the modified dialysis method, which was shown as following: 0.5 mL of honokiol-loaded PCEC micelles solution was placed in a tube covered with a dialysis membrane (molecular weight cutoff is 8–10 kDa and the dialysis area is 1 cm^2), and 0.5 mL of honokiol solution in DMSO (1 mg/mL) was used as control. The dialysis tubes were incubated in 20 mL of PBS (pre-warmed to 37 °C, pH 7.4) containing Tween80 (0.5%) at 37 °C with gentle shaking, and the media were displaced by pre-warmed fresh PBS at predetermined time. The released drug was quantified using reverse-phase High Performance Liquid Chromatography (RP-HPLC) with a C18 column (4.6 mm × 150 mm–5 μm, Sunfire Analysis column), with acetonitrile/water (60/40, v/v) as eluent solution.

2.7. Cell viability assay

A549 cells were plated at a density of 1×10^4 cells per well in 100 μL of DMEM medium in 96-well plates and grown for 48 h. The cells were then exposed to a series of honokiol-loaded PCEC micelles at different concentrations for 48 h, and 0.5 mL of honokiol solution in DMSO (1 mg/mL) was used as control. Then, viability of cells was measured using the methylthiazoletetrazolium (MTT) method. Briefly, the mean percentage of cell survival relative to that of untreated cells was estimated from data from six individual experiments. The concentration of samples at which cell killing was 50% (IC_{50}) was calculated by curve fitting using SPSS software.

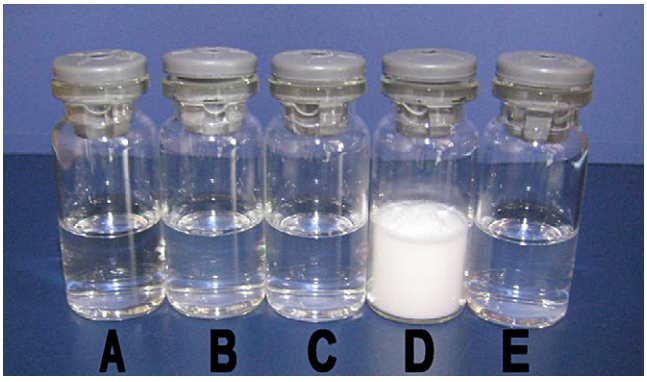

Fig. 1. Photographs of several solutions. (A) Water, at 25 °C; (B) blank PCEC micelles at the concentration of 10 mg/ml(w/v), at 25 °C; (C) honokiol-loaded PCEC micelles at the concentration of 10 mg/ml(w/v), at 25 °C; (D) Freeze-dried honokiol-loaded micelles at the concentration of 10 mg/ml(w/v), at 4 °C; (E) Re-dispersed freeze-dried honokiol-loaded micelles at the concentration of 10 mg/ml(w/v), at 25 °C.

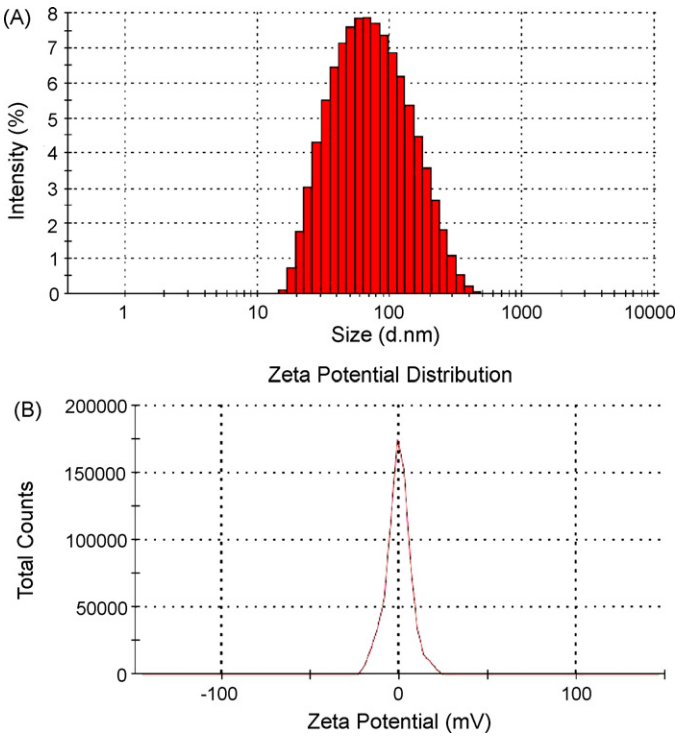

Fig. 2. Particle size (61 nm) and zeta potential (−0.502 mV) distribution spectra of prepared HK-loaded PCEC micelles.

3. Result

3.1. Characterization of honokiol-loaded micelles

Honokiol-loaded micelles in the present study were prepared by self-assembly of triblock PCEC copolymer. During the preparation of honokiol micelles, we were able to adjust the drug loading and encapsulation efficiency by varying the honokiol/PCEC weight ratio. With increase in honokiol content, a reasonable increase in drug loading was observed, leading to a decrease in encapsulation efficiency (Table 1), which might be because the dissolved PCEC was not enough to coat and stabilize more amounts of honokiol micelles.

Table 1
Drug loading and encapsulation efficiency of the obtained micelles at various weight ratio.

Code	HK/PCEC (w/w)	Drug loading (%)	Encapsulation efficiency (%)
S1	1:20	2.7	53.6
S2	2:20	4.8	50.0
S3	3:20	6.4	44.0

The amount of HK in the solution was determined by HPLC.

The appearance of prepared honokiol micelles suspension was shown in Fig. 1 and the clear solution could be observed. We chose the honokiol-loaded micelles with HK/PCEC of 2:20 and the obtained micelles were characterized in detail. The average particle size of obtained honokiol micelle was about 61 nm (Fig. 2A). Also, the zeta potential of prepared honokiol micelles was −0.502 mV and zeta potential distribution spectrum could be seen in Fig. 2B. Meanwhile, Fig. 3 shows the AFM image of the prepared micelles. The AFM image revealed that PCEC micelle is flattned round species, thus confirming the spherical shape of micelles in solution. The diameters of the polymeric micelles observed by AFM were in good agreement with the determination of the particle size.

3.2. Crystallographic study

Crystallographic assay was performed by XRD and the result was presented in Fig. 4. In comparison with XRD diagram of honokiol, the absence of specific diffraction peak in diagram of HK-loaded micelle indicated that honokiol was amorphously encapsulated within their core-shell structure, thus forming stable micelles in aqueous media.

3.3. Thermo-sensitivity

The thermo-sensitive property of blank PCEC micelles in aqueous solution was shown in Fig. 5. The particle size of PCEC micelles decreased accordingly with rise in temperature from 4 °C to 50 °C. And at 37 °C, the particle size reached an average diameter around 150 nm. Hence, the size of prepared micelle offers the possibility for the application to the body via direct injection into the blood circulation, making them potential for therapeutics delivery carrier.

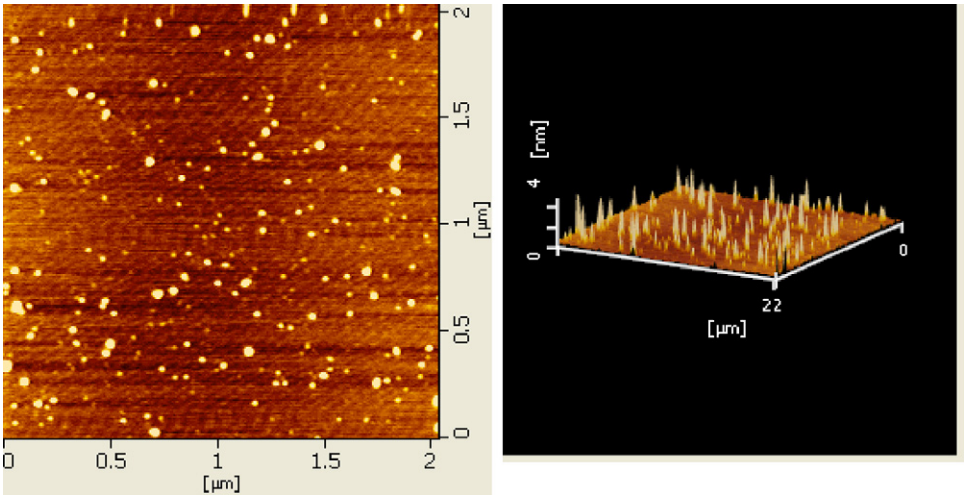

Fig. 3. The AFM image of prepared HK-loaded PCEC micelles.

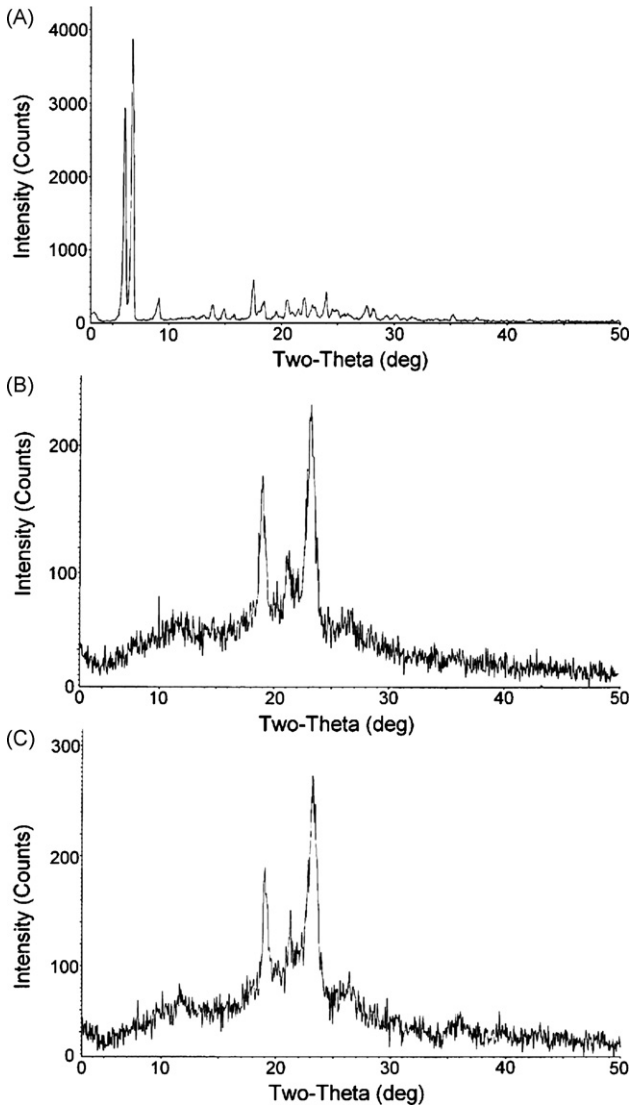

Fig. 4. X-ray diffraction spectra. (A) Honokiol crystal; (B) blank PCEC micelles; and (C) prepared honokiol-loaded PCEC micelles.

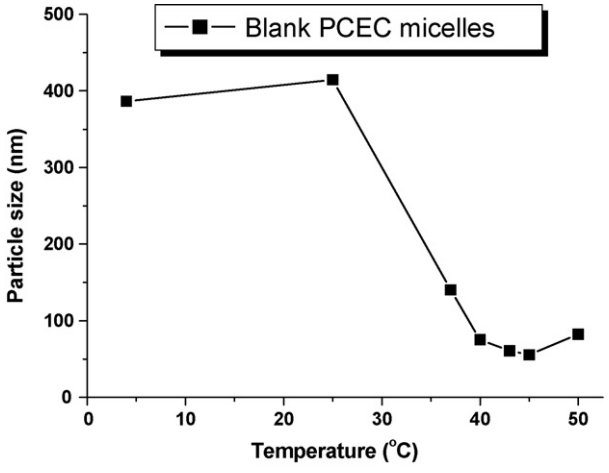

Fig. 5. Effect of temperature on particle size of blank PCEC micelles. PCEC micelles at the concentration of 10% (w/w) were measured at various temperature. All results were the mean of three test runs, each after an equilibration for 30 min. The particle size shrank while temperature rises from 4 °C to 50 °C.

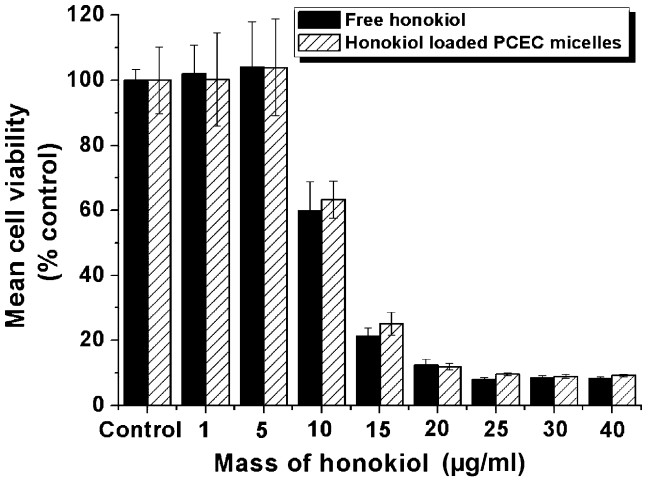

Fig. 6. Cell viability of lung cancer A549 cells. A549 cells were exposed to different concentrations of free honokiol or HK-loaded micelles for 48 h, respectively.

3.4. The cytotoxicity of honokiol-loaded micelles

The MTT assay was performed to evaluate the toxicity of drug-loaded polymeric micelles and free HK to investigate whether micelles influenced the cytotoxity of honokiol. Both free HK and HK-loaded micelles at various concentrations significantly decreased the viability of A549 cells in a dose-dependent manner. Fig. 6 shows the influence of drug loading on cell viability. The result indicates that the cytotoxicity of HK-loaded micelles is comparable to that of free HK.

3.5. In vitro release behavior of honokiol from PCEC micelles

Fig. 7 shows the release profiles of free HK and HK-loaded PCEC micelles in PBS (pH 7.4, 37 °C). In comparison to free HK, a typical two-phase-release profile of HK-loaded PCEC micelles was observed. That is, a relatively rapid release in the first stage followed by a sustained and slow release over a prolonged time up to several weeks. It was found that only 23% HK released from PCEC micelles within 24 h, while free HK released about 100% into the outside media.

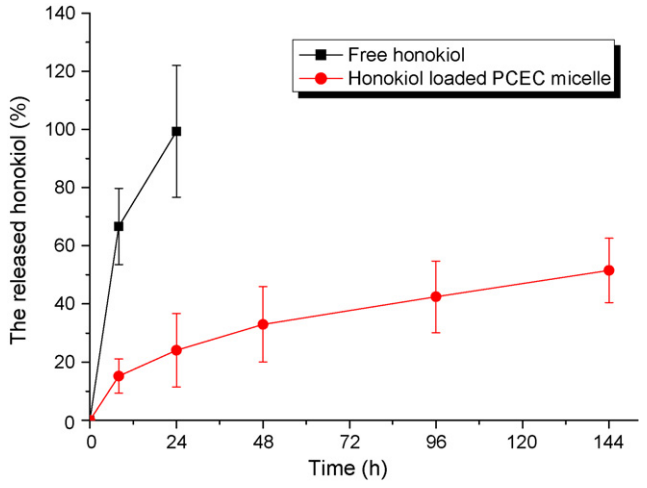

Fig. 7. Drug release profiles of free HK and HK-loaded micelles in PBS solution at pH 7.4.

4. Discussion

Various micelles occupy a huge area of research because they offer a vast range of possibilities for the use as vehicles for enzyme encapsulation (Chang and Prakash, 2001), gene therapy (Wang et al., 2007; Kim et al., 2008), biosensors (Cao et al., 2002; Demers et al., 2002), and mostly, hydrophobic drug delivery (Carstens et al., 2008; Kim et al., 2001a; Sant et al., 2005). It has been reported that paclitaxel (Genexol)-contained biodegradable polymeric micellar system (Genexol-PM) was newly developed by using monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide) (mPEG-PDLLA) and paclitaxel (Hamaguchi et al., 2007). Also, novel SN-38–incorporated polymeric micelles, NK012, to eradicate Vascular endothelial growth factor-secreting bulky tumors (Koizumi et al., 2006) and cisplatin-incorporated polymeric micelles which can eradicate solid tumors in mice have been successfully prepared (Nishiyama et al., 2003). Recently, TNP-470, an inhibitor of angiogenesis, was conjugated to monomethoxy-poly(ethylene glycol)-polylactic acid (mPEG-PLA) to form polymeric micelles, using the oral route of administration, and inhibited the tumor growth in mice (Benny et al., 2008). Until now, several micellar formulations of antitumor drugs have been intensively studied in preclinical and clinical trials (Hamaguchi et al., 2007; Nakanishi et al., 2001; Kim et al., 2004). In addition, the other polymeric micelles such as poly(ethylene oxide)-*b*-poly[(*R*)-3-hydroxy butyrate]-*b*-poly(ethylene oxide) PEO-PHB-PEG (Li et al., 2003), polyethylene glycol-phosphatidyl ethanolamine (PEG-PE) (Sawant et al., 2008), poly(ethylene glycol)-block-poly(aspartic acid) (PEG-PAA) (Kakizawa et al., 2004), and etc, are also promising nanocarriers for drug or gene delivery.

Several observation were made in the present study concerning PCEC, honokiol and self-assembly. We have successfully prepared the honokiol micelles with small particle size, stable drug loading and sustained drug release behavior by self-assembly. The average diameter of honokiol-loaded micelles was 61 nm, and the zeta potential (−0.502 mV) implied a nearly neutral surface charge. These, in combination with nanostructure of the micelles observed by AFM, suggested that the prepared micelles were stable and could be well-dispersed in water (Fig. 1). The X-ray diffraction analysis revealed the micro-domain structure of honokiol in the polymeric micelles. The spectrum showed a transformation of honokiol from crystalline structure to amorphous micelles. Honokiol may be suspended in water to form polymeric micelles due to amphiphilicity of PCEC copolymers, hence, amorphous honokiol molecules are essentially locked in the core-shell structure. Moreover, cytotoxicity results revealed that the honokiol-loaded micelles remained the comparable anticancer effect even though honokiol was released in an extended behavior. In comparison with free honokiol, the much slower release of honokiol from PCEC micelles can be attributed to the molecular structural characteristics of polymeric micelles. During the process, honokiol was first released inside the hydrophobic core region of polymeric micelles because honokiol was attached to PCL comprising the core region, and honokiol then diffused out from the micelle, eventually, into the incubation medium. This delay of drug release indicates their potential applicability in drug carrier to minimize the exposure of healthy tissues while increasing the accumulation of therapeutic drug in the tumor site.

Amphiphilic molecules in water are the most studied example of self-assembling molecules in selective solvents. The co-solvent evaporation method and dialysis method (Huh et al., 2005) were intensively studied to form polymeric micelles. However during the formation, organic solvent, such as methanol (Qiu and Bae, 2007), acetone (Peng et al., 2008b), or dimethylformamide (DMF) (Kim et al., 2001b; Park et al., 2005; Hu et al., 2003) are widely used as the solvent for drugs and polymeric materials, eventually followed by removal of organic solvents using evaporating, freeze drying, or dialysis. To the best of our knowledge, little work has been done to prepare polymeric micelles by dissolving copolymers in distilled water and heating without any other organics. This method could be highly welcome in preparation of micelles due to absence of toxicity organic solvents. Therefore, formulation of polymeric micelles triggered by heating in distilled water could ensure the safety of micelles both in the formation process and further clinical use.

Honokiol is a small molecule that has been demonstrated to have antiangiogenic and antitumor properties in diverse tumors (Yang et al., 2002; Fong et al., 2005; Hibasami et al., 1998). Recently, some studies have revealed the remarkable antitumor effects of honokiol on human ovarian tumor SKOV3 and COC1 cells (Liu et al., 2008), lung cancer CH27 cells (Yang et al., 2002), leukemia HL-60 cells (Fong et al., 2005), Molt 4B cells (Hibasami et al., 1998), etc. However, the application of honokiol is limited by its poor solubility in water. In this study we have successfully prepared honokiol-loaded micelles to increase the solubility of hydrophobic therapeutic agents in water. Meanwhile, PCEC, a biodegradable triblock copolymer with good biocompatibility, was adopted as drug carrier (Zhou et al., 2003; Jia et al., 2008; Huang et al., 2004). The findings in the present study demonstrate that honokiol can be core-encapsulated in the micelles to form a stable particle with sustained release behavior. Honokiol-loaded PCEC micelles also have comparable cytotoxicity with free honokiol. These properties of honokiol-loaded micelle suggest that it may be a potential new dosage form to deliver hydrophobic antitumor drugs.

5. Conclusion

In this study, we have successfully prepared honokiol-loaded PCEC micelles in a novel method without any organic solvent. We were able to control the drug loading and encapsulation efficiency by varying the weight ratio of HK and PCEC. The particle size, zeta potential and AFM image of the prepared micelle indicated its stability and solubility. Moreover, XRD diagram suggested that honokiol was amorphously enclosed in the core-shell architecture. The encapsulation of honokiol led to sustained release of the micelles up to several weeks while the cytotoxicity remained comparable to that of free honokiol. The PCEC micelles could be well-dispersed in water, thus increasing the solubility of honokiol, hence, providing the possibility for a new dosage form.

References

Benny, O., Fainaru, O., Adini, A., Cassiola, F., Bazinet, L., Adini, I., Pravda, E., Nahmias, Y., Koirala, S., Corfas, G., D'Amato, R.J., Folkman, J., 2008. An orally delivered small-molecule formulation with antiangiogenic and anticancer activity. Nat. Biotechnol. 26, 799–807.

Cai, S., Vijayan, K., Cheng, D., Lima, E.M., Discher, D.E., 2007. Micelles of different morphologies—advantages of worm-like filomicelles of PEO-PCL in paclitaxel delivery. Pharm. Res. 24, 2099–2109.

Cao, Y.W.C., Jin, R.C., Mirkin, C.A., 2002. Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. Science 297, 1536–1540.

Carstens, M.G., de Jong, P.H., van Nostrum, C.F., Kemmink, J., Verrijk, R., de Leede, L.G., Crommelin, D.J., Hennink, W.E., 2008. The effect of core composition in biodegradable oligomeric micelles as taxane formulations. Eur. J. Pharm. Biopharm. 68, 596–606.

Chang, T.M.S., Prakash, S., 2001. Procedures for microencapsulation of enzymes, cells and genetically engineered microorganisms. Mol. Biotechnol. 17, 249–260.

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

Choi, C.Y., Chae, S.Y., Nah, J.W., 2006. Thermosensitive poly(N-isopropylacrylamide)-*b*-poly(ε-caprolactone) nanoparticles for efficient drug delivery system. Polymer 47, 4571–4580.

Croy, S.R., Kwon, G.S., 2006. Polymeric micelles for drug delivery. Curr. Pharm. Des. 12, 4669–4684.

Demers, L.M., Ginger, D.S., Park, S.J., Li, Z., Chung, S.W., Mirkin, C.A., 2002. Direct patterning of modified oligonucleotides on metals and insulators by dip–pen nanolithography. Science 296, 1836–1838.

Dikalov, S., Losik, T., Arbiser, J.L., 2008. Honokiol is a potent scavenger of superoxide and peroxyl radicals. Biochem. Pharmacol. 76, 589–596.

Fong, W.F., Tse Anfernee, K.W., Poon, K.H., Wang, C., 2005. Magnolol and honokiol enhance HL-60 human leukemia cell differentiation induced by 1.25- dihydroxyvitamin D3 and retinoic acid. Int. J. Biochem. Cell. Biol. 37, 427–441.

Gou, M.L., Dai, M., Li, X.Y., Wang, X.H., Gong, C.Y., Xie, Y., Wang, K., Zhao, X., Qian, Z.Y., Wei, Y.Q., 2008a. Preparation and characterization of honokiol nanoparticles. J. Mater. Sci.: Mater. Med. 19, 2605–2608.

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., 2008b. Preparation and characterization of magnetic poly(ε-caprolactone)-poly(ethyleneglycol)-poly(ε-caprolactone) (PCEC) microspheres. J. Mater. Sci.: Mater. Med. 19, 1033–1041.

Hamaguchi, T., Kato, K., Yasui, H., Morizane, C., Ikeda, M., Ueno, H., Muro, K., Yamada, Y., Okusaka, T., Shirao, K., Shimada, Y., Nakahama, H., Matsumura, Y., 2007. A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. Br. J. Cancer 97, 170–176.

Hibasami, H., Achiwa, Y., Katsuzaki, H., Imai, K., Yoshioka, K., Nakanishi, K., Ishii, Y., Hasegawa, M., Komiya, T., 1998. Honokiol induces apoptosis in human lymphoid leukemia Molt 4B cells. Int. J. Mol. Med. 2, 671–673.

Hu, Y., Jiang, X., Ding, Y., Zhang, L., Yang, C., Zhang, J., Chen, J., Yang, Y., 2003. Preparation and drug release behaviors of nimodipine-loaded poly(caprolactone) poly(ethylene oxide) polylactide amphiphilic copolymer nanoparticles. Biomaterials 24, 2395–2404.

Huang, M.H., Li, S., Hutmacher, D.W., Schantz, J.T., Vacanti, C.A., Braud, C., Vert, M., 2004. Degradation and cell culture studies on block copolymers prepared by ring opening polymerization of epsilon-caprolactone in the presence of poly(ethylene glycol). J. Biomed. Mater. Res. A 69, 417–427.

Huh, K.M., Lee, S.C., Cho, Y.W., Lee, J., Jeong, J.H., Park, K., 2005. Hydrotropic polymer micelle system for delivery of paclitaxel. J. Control. Release 101, 59–68.

Ideta, R., Tasaka, F., Jang, W.D., Nishiyama, N., Zhang, G.D., Harada, A., Yanagi, Y., Tamaki, Y., Aida, T., Kataoka, K., 2005. Nanotechnology-based photodynamic therapy for neovascular disease using a supramolecular nanocarrier loaded with a dendritic photosensitizer. Nano Lett. 5, 2426–2431.

Jang, W.D., Nakagishi, Y., Nishiyama, N., Kawauchi, S., Morimoto, Y., Kikuchi, M., Kataoka, K., 2006. Polyion complex micelles for photodynamic therapy: incorporation of dendritic photosensitizer excitable at long wavelength relevant to improved tissue-penetrating property. J. Control. Release 113, 73–79.

Jia, W.J., Gu, Y.C., Gou, M.L., Dai, M., Li, X.Y., Kan, B., Yang, J.L., Song, Q.F., Wei, Y.Q., Qian, Z.Y., 2008. Preparation of biodegradable polycaprolactone/poly(ethylene glycol)/polcaprolactone (PCEC) nanoparticles. Drug Delivery 15, 409–416.

Kakizawa, Y., Furukawa, S., Kataoka, K., 2004. Block copolymer-coated calcium phosphate nanoparticles sensing intracellular environment for oligodeoxynucleotide and siRNA delivery. J. Control. Release 97, 345–356.

Kim, S.C., Kim, D.W., Shim, Y.H., Bang, J.S., Oh, H.S., Wan Kim, S., Seo, M.H., 2001a. In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy. J. Control. Release 72, 191–202.

Kim, S.Y., Lee, Y.M., Shin, H.J., Kang, J.S., 2001b. Indomethacin-loaded methoxy poly(ethylene glycol)/poly(ε-caprolactone) diblock copolymeric nanosphere: pharmacokinetic characteristics of indomethacin in the normal Sprague–Dawley rats. Biomaterials 22, 2049–2056.

Kim, T.Y., Kim, D.W., Chung, J.Y., Shin, S.G., Kim, S.C., Heo, D.S., Kim, N.K., Bang, Y.J., 2004. Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies. Clin. Cancer Res 10, 3708–3716.

Kim, S.H., Jeong, J.H., Lee, S.H., Kim, S.W., Park, T.G., 2008. Local and systemic delivery of VEGF siRNA using polyelectrolyte complex micelles for effective treatment of cancer. J. Control. Release 129, 107–116.

Koizumi, F., Kitagawa, M., Negishi, T., Onda, T., Matsumoto, S., Hamaguchi, T., Matsumura, Y., 2006. Novel SN-38-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors. Cancer Res. 66, 10048–10056.

Kopecek, J., Kopeckova, P., Minko, T., Lu, Z.R., Peterson, C.M., 2001. Water soluble polymers in tumor targeted delivery. J. Control. Release 74, 147–158.

Li, J., Li, X., Ni, X.P., Leong, K.W., 2003. Synthesis and characterization of new biodegradable amphiphilic poly(ethylene oxide)-*b*-poly[(r)-3-hydroxy butyrate]-*b*-poly(ethylene oxide) triblock copolymers poly(ethylene oxide)-*b*-poly[(*R*)-3-hydroxy butyrate]-*b*-poly(ethylene oxide). Macromolecules 36, 2661–2667.

Li, Z., Liu, Y., Zhao, X., Pan, X., Yin, R., Huang, C., Chen, L., Wei, Y., 2008. Honokiol, a natural therapeutic candidate, induces apoptosis and inhibits angiogenesis of ovarian tumor cells. Eur. J. Obstet. Gynecol. Reprod. Biol. 140, 95–102.

Lin, J., Zhang, S., Chen, T., Lin, S., Jin, H., 2006. Micelle formation and drug release behavior of polypeptide graft copolymer and its mixture with polypeptide block copolymer. Int. J. Pharm. 336, 49–57.

Liu, H., Zang, C., Emde, A., Planas-Silva, M.D., Rosche, M., Kühnl, A., Schulz, C.O., Elstner, E., Possinger, K., Eucker, J., 2008. Anti-tumor effect of honokiol alone and in combination with other anti-cancer agents in breast cancer. Eur. J. Pharmacol. 591, 43–51.

Nakanishi, T., Fukushima, S., Okamoto, K., Suzuki, M., Matsumura, Y., Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 2001. Development of the polymer micelle carrier system for doxorubicin. J. Control. Release 74, 295–302.

Nakayama, M., Chung, J.E., Miyazaki, T., Yokoyama, M., Sakai, K., Okano, T., 2007. Thermal modulation of intracellular drug distribution using thermoresponsive polymeric micelles. React. Funct. Polym. 67, 1398–1407.

Nishiyama, N., Okazaki, S., Cabral, H., Miyamoto, M., Kato, Y., Sugiyama, Y., Nishio, K., Matsumura, Y., Kataoka, K., 2003. Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice. Cancer Res. 63, 8977–8983.

Park, E.K., Lee, S.B., Lee, Y.M., 2005. Preparation and characterization of methoxy poly(ethylene glycol)/poly(e-caprolactone) amphiphilic block copolymeric nanospheres for tumor-specific folate-mediated targeting of anticancer drugs. Biomaterials 26, 1053–1061.

Peng, C.L., Shieh, M.J., Tsai, M.H., Chang, C.C., Lai, P.S., 2008a. Self-assembled star-shaped chlorin-core poly(ε-caprolactone)–poly(ethyleneglycol) diblock copolymer micelles for dual chemo-photodynamic therapies. Biomaterials 29, 3599–3608.

Peng, C.L., Shieh, M.J., Tsai, M.H., Chang, C.C., Lai, P.S., 2008b. Self-assembled star-shaped chlorin-core poly(varepsilon-caprolactone)-poly(ethylene glycol) diblock copolymer micelles for dual chemo-photodynamic therapies. Biomaterials 29, 3599–3608.

Qiu, L.Y., Bae, Y.H., 2007. Self-assembled polyethylenimine-graft-poly(ε-caprolactone) micelles as potential dual carriers of genes and anticancer drugs. Biomaterials 28, 4132–4142.

Sant, V.P., Smith, D., Leroux, J.C., 2005. Enhancement of oral bioavailability of poorly water-soluble drugs by poly(ethylene glycol)-block-poly(alkyl acrylate-co-methacrylic acid) self-assemblies. J. Control. Release 104, 289–300.

Sawant, R.R., Sawant, R.M., Torchilin, V.P., 2008. Mixed PEG-PE/vitamin E tumor-targeted immunomicelles as carriers for poorly soluble anti-cancer drugs: improved drug solubilization and enhanced in vitro cytotoxicity. Eur. J. Pharm. Biopharm. 70, 51–57.

Shuai, X.T., Merdan, T., Unger, F., Wittmar, M., Kissel, T., 2003. Novel biodegradable ternary copolymers hy-PEI-g-PCL-*b*-PEG: synthesis, characterization, and potential as efficient nonviral gene delivery vectors. Macromolecules 36, 5751–5759.

Takeuchi, Y., Ichikawa, K., Yonezawa, S., Kurohane, K., Koishi, T., Nango, M., Namba, Y., Oku, N., 2004. Intracellular target for photosensitization in cancer antiangiogenic photodynamic therapy mediated by polycation liposome. J. Control. Release 97, 231–240.

Vargas, A., Pegaz, B., Debefve, E., Konan-Kouakou, Y., Lange, N., Ballini, J.P., van den Bergh, H., Gurny, R., Delie, F., 2004. Improved photodynamic activity of porphyrin loaded into nanoparticles: an in vivo evaluation using chick embryos. Int. J. Pharm. 286, 131–145.

Wang, Y., Wang, L.S., Goh, S.H., Yang, Y.Y., 2007. Synthesis and characterization of cationic micelles self-assembled from a biodegradable copolymer for gene delivery. Biomacromolecules 8, 1028–1037.

Yang, S.E., Hsieh, M.T., Tsai, T.H., Hsu, S.L., 2002. Down-modulation of Bcl-XL, release of cytochrome c and sequential activation of caspases during honokiol-induced apoptosis in human squamous lung cancer CH27 cells. Biochem. Pharmacol. 63, 1641–1651.

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