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Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL–PEG–PCL) nanoparticles for honokiol delivery *in vitro*☆

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ABSTRACT

In this article, poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCL–PEG–PCL, PCEC) nanoparticles were successfully prepared for honokiol delivery *in vitro*. Blank or honokiol loaded PCL–PEG–PCL nanoparticles were prepared in moderate condition by solvent diffusion method without using any surfactants. The prepared blank PCL–PEG–PCL nanoparticles are mono-dispersed and smaller than 200 nm. The particle size increased with increase in polymer concentration and oil–water (O/W) ratio. The prepared PCL–PEG–PCL nanoparticles (40 mg/mL, ca. 106 nm) did not induce hemolysis *in vitro*. And the 50% inhibiting concentration (IC50) of it (48 h) on HEK293 cells was higher than 5 mg/mL. Honokiol could be efficiently loaded into PCL–PEG–PCL nanoparticles and released from these nanoparticles in an extended period *in vitro*. After honokiol (HK) was entrapped into PCL–PEG–PCL nanoparticles, the particle size increased with the increase in HK/PCEC mass ratio in feed, and the encapsulated honokiol retained potent anticancer activity *in vitro*. The PCL–PEG–PCL nanoparticle was suitable for honokiol delivery, and such honokiol loaded PCL–PEG–PCL nanoparticle was a novel honokiol formulation.

1. Introduction

Honokiol, 3′,5-di-2-propenyl-1,1′-biphenyl-2,4′-diol, is a constituent of Chinese medicinal herb *Magnolia officinalis/grandiflora*. Honokiol has a variety of pharmacological effects, such as anti-inflammatory (Liou et al., 2003a,b), anti-thrombotic (Teng et al., 1988), anti-arrhythmic (Liou et al., 2003a,b), anti-oxidative (Lo et al., 1994), central depressant, muscle relaxant and anxiolytic effects (Maruyama and Kuribara, 2000). In the past decades, many researches suggested that honokiol had anticancer activity and showed great potential application in cancer treatment. Honokiol could inhibit skin tumor promotion (Konoshima et al., 1991), inhibit angiogenesis and tumor growth *in vivo* (Bai et al., 2003), induce caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells (Battle et al., 2005), treat cisplatin-resistant human ovarian cancer (Luo et al., 2008), overcome conventional drug resistance in human multiple myeloma by induction of caspase-dependent and -independent apoptosis (Ishitsuka et al., 2005) and down-regulate of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR (Xu et al., 2006). Meanwhile, combined with Adriamycin or caspase, honokiol also showed synergistic antitumor effects (Hou et al., 2008; Jiang et al., 2008). Although honokiol has great anticancer activity, its wider application was limited due to its very poor solubility in water. Therefore, it is interesting to develop a novel formulation for honokiol.

Since Nobel Laureate Richard Feynman predicted the emergence of a new science called nanotechnology in 1959, nanotechnology has played a very important role in many fields, and has shown great potential application in cancer therapy (Wang et al., 2008; Service, 2005; Ferrari, 2005; Alexis et al., 2008). In the past decades, advanced anticancer drug delivery systems based on biodegradable polymeric nanoparticles attracted extensive research. Some anticancer agents based on nano-vectors had moved into clinical trial period (Wang et al., 2008; Ferrari, 2005; Alexis et al., 2008; Fassas

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and Anagnostopoulos, 2005; Allen and Cullis, 2004; Soepenberg et al., 2005; Ochi et al., 2005; Park et al., 2008). To overcome its poor water solubility, hydrophobic drug was entrapped into amphiphilic polymeric nanoparticles, and the nanoparticles could be well dispersed in water to meet the requirement of intravenous injection. It is known that paclitaxel shows significant activity against a wide range of cancers such as breast cancer, ovarian cancer, lung cancer and head and neck carcinomas (Arbuck and Blaylock, 1995). But the success of clinical application of paclitaxel is mainly limited by its low therapeutic index and low solubility in water and many other acceptable pharmaceutical solvents. Cremophor ELs, the additive in the only commercially available taxol, has shown severe anaphylaxis in patients (Weiss et al., 1990). Now, albumin–paclitaxel, HPMA copolymer–paclitaxel and paclitaxel poliglumex nanoparticles are already in clinical trial period. It is also suggested that the nano-paclitaxel is better than the traditional paclitaxel in clinic (Wang et al., 2008; Gradishar, 2005; Gradishar et al., 2005; Gradishar, 2006; Boddy et al., 2005; Meerum Terwogt et al., 2001). So, it is an attractive method to make a formulation based on nano-vector for hydrophobic drugs (Saffie-Sieverb et al., 2005). After drug loaded nanoparticle is injected into body, drug can be released in a controlled manner from the nanoparticle, which always results in the improvement of pharmacokinetics (Owens and Peppas, 2006). Moreover, nanoparticles can be passively targeted to solid tumor due to the enhanced permeability and retention (EPR) effect, which results in higher drug concentration in tumor that make anticancer drug more efficient and safer (Northfelt et al., 1996; Tang et al., 2007). It is also suggested that nano-vector has great potential application in overcoming multi-drug resistance (MDR) effect (Amiji et al., 2006; Vauthier et al., 2003; Zhang et al., 2008). Therefore, it is attractive to employ biodegradable nanoparticles to load anticancer drug to form nano-drug, which can overcome poor water solubility of hydrophobic drug and make drug more effective and safer than free drug.

Due to its biodegradability, good biocompatibility, low toxicity, amphiphilic property and ease to produce, the PCL–PEG–PCL copolymer is a novel candidate for injectable drug delivery system (Ge et al., 2002; Gou et al., 2008a). Previously, we synthesized PCL–PEG–PCL copolymer by ring-opening polymerization and prepared PCL–PEG–PCL nanoparticles by emulsion solvent evaporation method (Gou et al., 2007; Gou et al., 2008b). When the widely used emulsion solvent evaporation method is employed to prepare biomedical polymer nanoparticles, surfactants (which always are unwanted) must be used and is difficult to be removed from the nanoparticles completely (Shakesheff et al., 1997). Here, blank and honokiol loaded mono-dispersed PCL–PEG–PCL (PCEC) nanoparticles were prepared in a moderate condition by solvent diffusion method without using any surfactants. The obtained honokiol loaded PCL–PEG–PCL nanoparticles with the drug loading of ca. 20% were mono-dispersed and smaller than 200 nm. Comparing with our previous reports about honokiol formulation based on polymeric nanoparticles, this paper described a novel method to prepare honokiol loaded PCL–PEG–PCL nanoparticles, and the obtained nanoparticles also have many advantages over previous formulations (Gou et al., 2008d,e; Wei et al., 2009). The prepared honokiol loaded PCL–PEG–PCL nanoparticles is a novel honokiol formulation and might be a novel anticancer agent for cancer therapy.

2. Experimental

2.1. Material

Poly(ethylene glycol) (PEG, $Mn = 4000$), ε-caprolactone (ε-CL), stannous octoate ($Sn(Oct)_2$), RPMI-1640, DMEM, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma (USA). Dimethyl sulfoxide (DMSO) was 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. Preparation of blank or honokiol loaded PCL–PEG–PCL nanoparticles

First, the PCL–PEG–PCL copolymer was synthesized by ring-opening polymerization of ε-caprolactone initiated by PEG4000 as shown in Fig. 1(a) (Gou et al., 2008c). The number molecular weight of the used PCL–PEG–PCL was about 17,500 calculated from ^{1}H-NMR spectrum (data not shown here). Then, honokiol loaded PCL–PEG–PCL nanoparticles were prepared by solvent diffusion method as shown in Fig. 1(b). Briefly, the PCL–PEG–PCL and honokiol were co-dissolved in DMSO to form organic phase. Then, the organic phase was dropped into water under moderate mechanical stirring. In about 10 min, with the diffusion of DMSO into water, amphiphilic PCL–PEG–PCL block copolymer self-assembled into nanoparticles and their hydrophobic core encapsulated honokiol in aqueous solution as presented in Fig. 1(c). At last, the obtained nanoparticles were centrifuged and washed three times to remove the residual DMSO. Otherwise, honokiol was not added when blank PCL–PEG–PCL nanoparticles was prepared according to the same method.

2.3. Study of the physicochemical properties of prepared nanoparticles

Particle size distribution of nanoparticles was determined by laser diffraction particle sizer (Nano-ZS, Malvern Instrument, UK). The zeta potential of honokiol loaded nanoparticles in water was measured by Malvern Zeta analyzer (Nano-ZS, Malvern Instrument, UK). The temperature was kept at 25 °C during measuring. And all results were the mean of 3 test runs.

The morphology of prepared nanoparticles was observed on a transmission electron microscope (TEM) (H-6009IV, Hitachi, Japan): 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 before observation.

Crystallographic assays were performed on honokiol powder, blank PCL–PEG–PCL nanoparticles, and honokiol loaded PCL–PEG–PCL nanoparticles by X-ray Diffractometer (XRD) (X'Pert Pro, Philips, Netherlands) using Mo Kα radiation.

The concentration of honokiol was determined by High Performance Liquid Chromatography (HPLC) Instrument (Waters Alliance 2695). Solvent delivery system was 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 C18 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.

Drug loading and entrapment efficiency was determined as follows. Briefly, 0.2 mL of drug loaded PCL–PEG–PCL nanoparticles was introduced 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 weight. Afterwards, the dried deposit was dissolved in 0.1 mL of DCM and diluted by acetonitrile. Meanwhile, the amount of honokiol in the solution was determined by HPLC. Drug loading (DL) and encapsulation efficiency (EE) of drug-loaded nanoparticles were calculated according

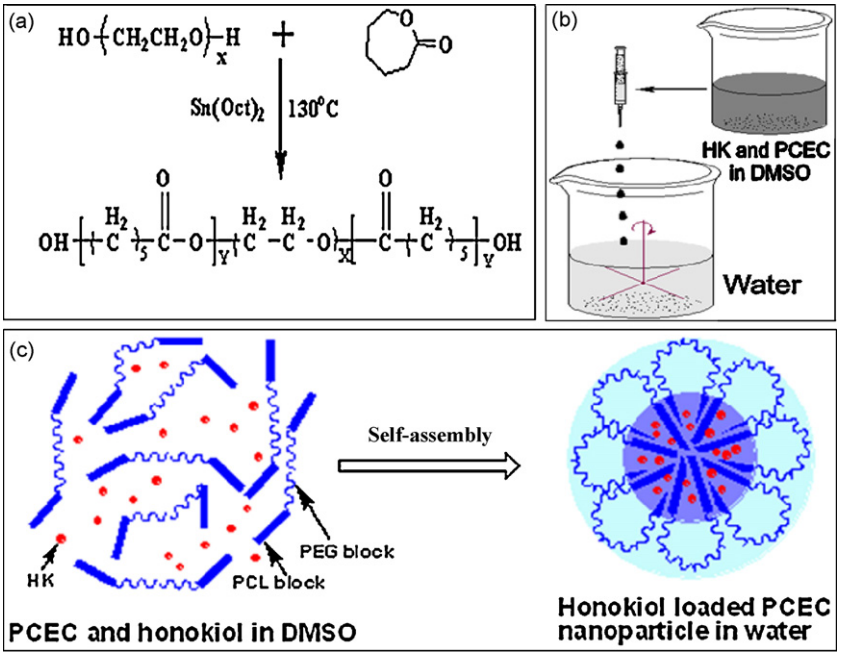

Fig. 1. Preparation of PCL–PEG–PCL (PCEC) nanoparticles containing honokiol (HK). (a) Synthesis of PCL–PEG–PCL copolymer by ring-opening polymerization of ε-caprolactone initiated by PEG4000; (b) preparation of PCL–PEG–PCL nanoparticles containing honokiol by solvent diffusion method; and (c) honokiol loaded PCL–PEG–PCL nanoparticles formed in water due to self-assembly.

to Eqs. (1) and (2):

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

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

2.4. Hemolytic test in vitro

The hemolytic study was performed on PCL–PEG–PCL nanoparticles *in vitro*. Briefly, 0.5 mL of sample at different concentrations in normal saline was diluted into 2.5 mL by normal saline and added into 2.5 mL of rabbit erythrocyte suspension (2%) in normal saline under 37 °C. Normal saline and distilled water were employed as negative and positive control, respectively. Three hours later, the erythrocyte suspension was centrifuged and the color of the supernatant was compared with the negative control. If the supernatant solution was absolute achromatic, it implied that there was not hemolysis. In contrast, hemolysis occurred when the supernatant solution was red.

2.5. In vitro release study

To determine the *in vitro* release kinetics of honokiol from nanoparticles, 0.5 mL of honokiol loaded PCL–PEG–PCL nanoparticles slurry was placed in a dialysis bag (molecular mass cutoff 10 kDa). The dialysis bags were incubated in 30 mL of phosphate buffer (pH 7.4) containing Tween80 (0.5%) at 37 °C with gentle shaking, and incubation medium were replaced by fresh medium at predetermined time points. The released drug was quantified, and the cumulative release profile with time was demonstrated. This study was repeated 3 times, and result was expressed as mean value ± S.D.

2.6. Analysis of cytotoxicity

All the studied cells were maintained in State Key Laboratory of Biotherapy, Sichuan University, China. The cytotoxicity of PCL–PEG–PCL nanoparticles was evaluated by cell viability assay on HEK293 cells. Briefly, HEK293 cells were plated at a density of 5×10^3 cells per well in 100 μL of DMEM medium in 96-well plates and grown for 24 h. The cells were then exposed to PCL–PEG–PCL nanoparticles at different concentration for 48 h, and the cells viability was measured using the methylthiazoletetrazolium (MTT) method. The cytotoxicity of honokiol or nanoparticle-encapsulated honokiol on cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) human ovarian cancer cells was also evaluated by cell proliferation assay. Briefly, A2780s or A2780cp cells were plated at a density of 5×10^3 cells per well in 100 μL of RPMI 1640 medium in 96-well plates and grown for 24 h. The cells were then exposed to free honokiol or nanoparticle-encapsulated honokiol at different concentrations for 48 h. The cell's viability was measured by the MTT method. These assays were repeated 6 times, and results were expressed as mean value ± S.D.

3. Results

3.1. Effect of process parameters on the particle size

Mono-dispersed PCL–PEG–PCL nanoparticles were prepared by solvent diffusion method in this work. The DMSO is a water soluble organic solvent, while PCL–PEG–PCL is water insoluble amphiphilic copolymer. When PCL–PEG–PCL organic solution in DMSO was dropped into water under stirring, the PCL–PEG–PCL organic phase was dispersed into small drops due to mechanical stirring and diffusion of DMSO. Then, as the continued diffusion of DMSO, PCL–PEG–PCL was precipitated in water and self-assembled into nanosphere with core-shell structure due to the amphiphilic property of PCL–PEG–PCL.

Effects of process parameters on the particle size of nanoparticles were studied, because the particle size would greatly affect the fate of nanoparticles *in vivo* and was one of the most important parameters of drug vectors. The PCL–PEG–PCL concentration in DMSO had great effect on the particle size of obtained nanoparticles. As shown in Fig. 2(a), particle size increased with increase in polymer concentration. But effect of temperature on the

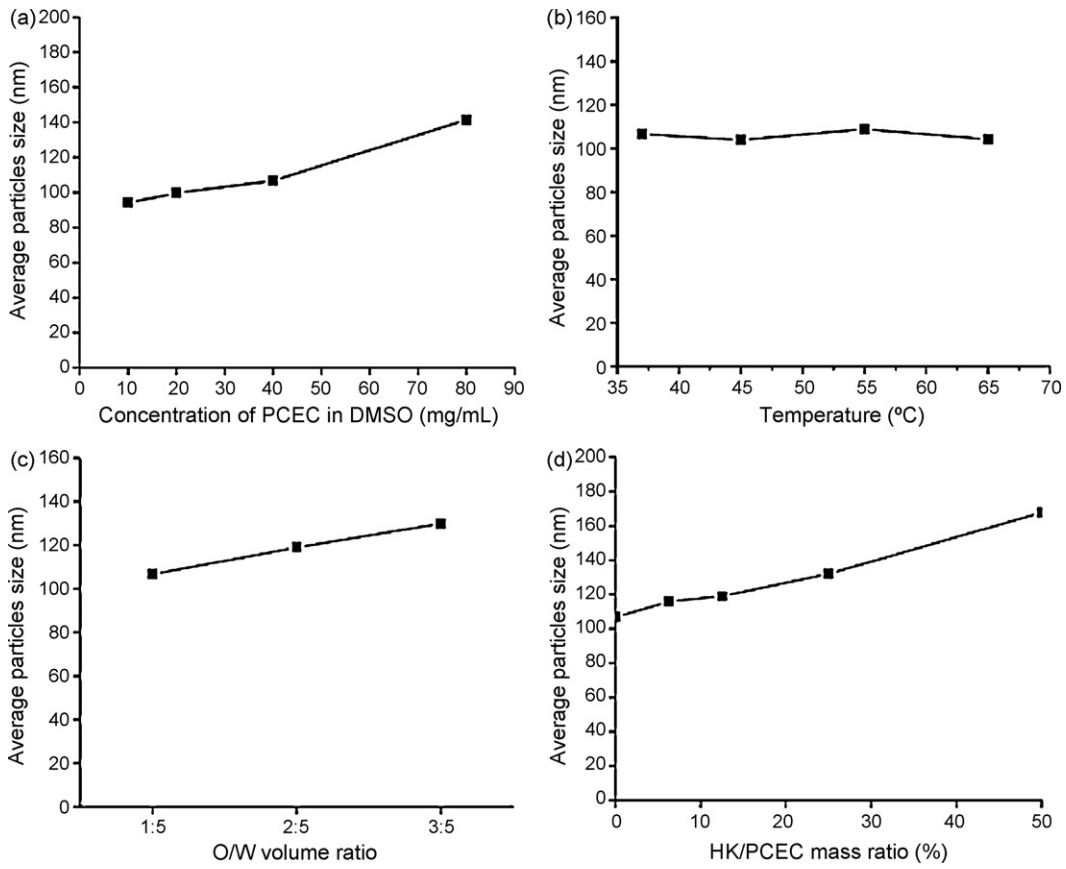

Fig. 2. Effects of process parameters on the average particle size of obtained nanoparticles in this study. (a) Effect of polymer concentration on average particle size of blank PCEC nanoparticles; (b) effect of temperature on average particle size of blank nanoparticles; (c) effect of O/W phase ratio on average particle size of blank PCEC nanoparticles; and (d) effect of HK/PCEC mass ratio in feed on the average particle size of honokiol loaded PCEC nanoparticles.

particle size was not observed when nanoparticle was prepared under the temperature between 37 °C and 65 °C as presented in Fig. 2(b). Meanwhile, the effect of oil–water (O/W) ratio on the particle size was studied, and it was indicated that particle size increased with increase in O/W ratio as shown in Fig. 2(c). When honokiol loaded PCL–PEG–PCL nanoparticles were prepared, particle size of honokiol loaded PCL–PEG–PCL nanoparticles increased with increase in drug/polymer mass ratio in feed as presented in Fig. 2(d). It was implied that honokiol loaded PCL–PEG–PCL nanoparticles with different particle size could be obtained by adjusting polymer concentration, O/W ratio and HK/PCEC mass ratio.

3.2. Safety evaluation of PCL–PEG–PCL nanoparticles in vitro

Hemolytic test was performed on PCL–PEG–PCL nanoparticles. As shown in Fig. 3, PCL–PEG–PCL nanoparticles at the concentration of 40 mg/mL did not cause any hemolysis on rabbit erythrocyte comparing with the negative control (normal saline). Meanwhile, the cytotoxicity of blank PCL–PEG–PCL was evaluated by cell viability assay on HEK293 cells as normal cells. According to Fig. 4, the 50% inhibiting concentration (IC_{50}) of PCL–PEG–PCL nanoparticle was higher than 5 mg/mL which suggested that PCL–PEG–PCL nanoparticle was very low toxic, exactly non-toxic. So, the prepared

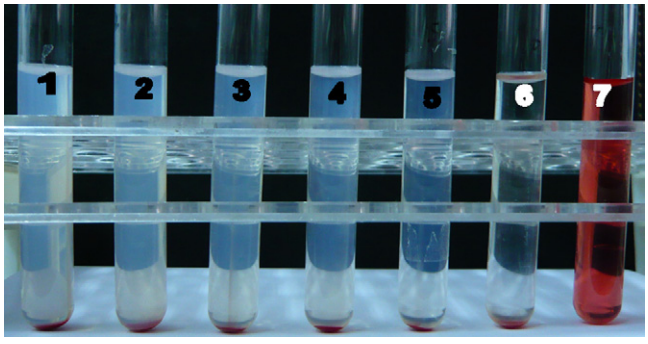

Fig. 3. Hemolytic test on the PCL–PEG–PCL nanoparticles. This image is taken at 3 h after reaction. The concentration of PCL–PEG–PCL nanoparticles is 40 mg/mL (1), 32 mg/mL (2), 24 mg/mL (3), 16 mg/mL (4) and 8 mg/mL (5). Sample (6) is normal saline used as negative control, and sample (7) is distilled water used as positive control.

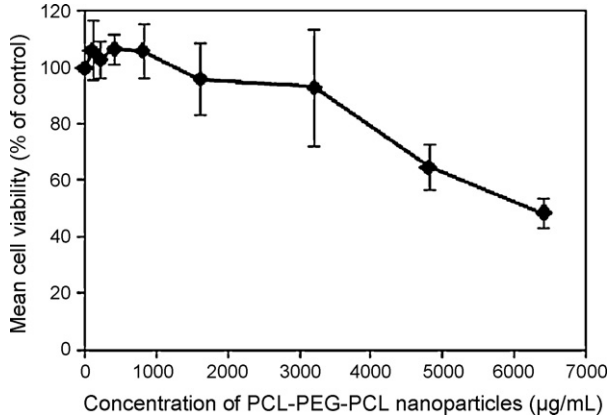

Fig. 4. Cytotoxicity of blank PCL–PEG–PCL nanoparticles with the mean particle size of 106 nm on HEK293 cells *in vitro*.

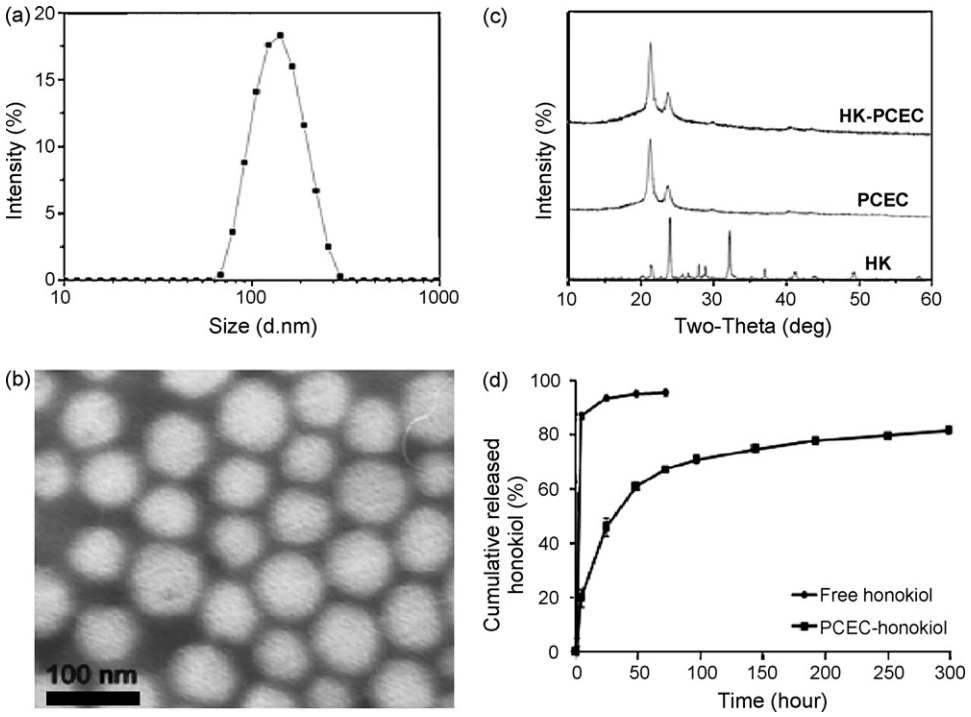

Fig. 5. Characterization of honokiol loaded PCL–PEG–PCL nanoparticles. (a) Size distribution spectrum; (b) TEM image; (c) XRD assays; and (d) drug release profile determined by a dialysis method *in vitro*.

PCL–PEG–PCL nanoparticle might be safe vector for intravenous injection.

3.3. Physical characterization of honokiol loaded PCL–PEG–PCL nanoparticle

Honokiol loaded PCL–PEG–PCL nanoparticle at the mean particle size of 132 nm (determined by laser diffraction size detector) with HK/PCEC mass ratio (in feed) of 25% was chosen for medical application and was characterized in detail. These honokiol loaded PCL–PEG–PCL nanoparticle had the encapsulation efficiency of ca. 99% and drug loading of ca. 20%. The particle size distribution spectrum of it was presented in Fig. 5(a) that was determined by laser diffraction size detector, and the polydispersity index (PDI) is 0.06, which suggest that the obtained honokiol loaded PCL–PEG–PCL nanoparticle was mono-dispersed and did not form aggregates in water. The zeta potential of the honokiol loaded PCL–PEG–PCL nanoparticles was −3.8 mV. Meanwhile, morphology study indicated that the prepared PCL–PEG–PCL nanoparticles containing honokiol had sphere appearance according to TEM photo as shown in Fig. 5(b). And according to the TEM image, the mean particle size of the honokiol loaded PCL–PEG–PCL nanoparticles was ca. 80 nm. Otherwise, crystallography study was performed on honokiol crystal, blank PCL–PEG–PCL nanoparticle and honokiol loaded PCL–PEG–PCL nanoparticle as presented in Fig. 5(c). It could be found that the characteristic diffraction peaks of honokiol dispersed in the spectrum of honokiol loaded PCL–PEG–PCL nanoparticle which implied that honokiol was molecularly incorporated in PCL–PEG–PCL nanoparticle. At last, the release profile *in vitro* was evaluated. A sustained release manner could be visibly observed when honokiol released from PCL–PEG–PCL nanoparticle as shown in Fig. 5(d). These physical properties indicated that the prepared honokiol loaded PCL–PEG–PCL nanoparticle was novel honokiol formulation which could meet the requirement of intravenous injection.

3.4. Anticancer activity of honokiol loaded PCL–PEG–PCL nanoparticle

The cell viability assays were performed to evaluate the anticancer activity of honokiol loaded PCL–PEG–PCL nanoparticles and free honokiol. Both free honokiol and honokiol-loaded nanoparticle significantly decreased the viability of both A2790s and A2780cp cells with increase in honokiol concentration. Fig. 6 showed the influence of drug concentration on cell viability. The results indicated that the cytotoxicity of honokiol loaded PCL–PEG–PCL nanoparticles was comparable to that of free honokiol. Honokiol loaded PCL–PEG–PCL nanoparticles had potent anticancer effect on both cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) human ovarian cancer cells *in vitro*.

4. Discussion

Biodegradable polymer nanoparticles have great potential application in drug delivery system. Poly(ε-caprolactone) is biodegradable and biocompatible, and poly(ε-caprolactone) nanoparticles have been studied as drug delivery system *in vitro* and *in vivo* for many years (Sinha et al., 2004). But pure hydrophobic PCL nanoparticles tend to form aggregates in water due to its high specific surface area and high surface energy duet to its great hydrophobicity of PCL. Meanwhile, hydrophobic nanoparticles are always quickly captured by reticuloendothelial system (RES). Fortunately, studies indicated that PEGylated nanoparticles do not tend to aggregate and could prolong the circulation time in blood, which are critical important for injectable drug delivery system (Otsuka et al., 2003). So, PEGylated PCL nanoparticle was designed as drug delivery system *in vitro* and *vivo* (Gref et al., 1995; Zhou et al., 2003). Usually, PEGylated PCL nanoparticles have a core-shell structure with hydrophobic PCL core and hydrophilic PEG shell (Hu et al., 2007). The hydrophobic core can act as a depot for many hydrophobic drugs, while hydrophilic PEG shell make

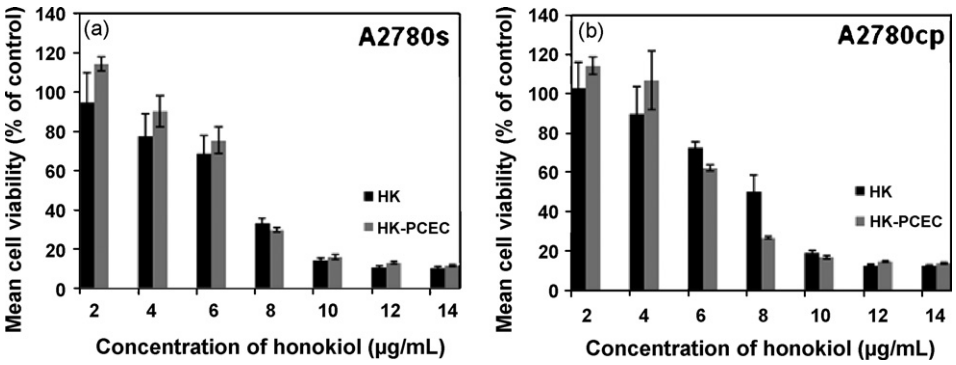

Fig. 6. Cytotoxicity of free honokiol and honokiol loaded PCL–PEG–PCL nanoparticles on human ovarian cancer cells. (a) Cytotoxicity on cisplatin-sensitive (A2780s) ovarian cancer cells; and (b) cytotoxicity on cisplatin-resistant (A2780cp) human ovarian cancer cells.

nanoparticle stable and long-circulating. In this paper, amphiphilic PCL–PEG–PCL nanoparticle was prepared and employed to load hydrophobic honokiol to overcome its poor solubility in water to meet the requirement of clinic administration. PCL–PEG–PCL nanoparticles were prepared by solvent diffusion method without using any unwanted surfactants, and effects of process parameters on particle size were discussed. The prepared PCL–PEG–PCL nanoparticles (40 mg/mL) did not induce hemolysis (Fig. 3) and showed non-cytotoxicity on HEK293 cells *in vitro* (Fig. 4), which implied that the PCL–PEG–PCL nanoparticle was safe for biomedical application. The obtained PCL–PEG–PCL nanoparticles containing honokiol were mono-dispersed and smaller than 200 nm, which could meet the requirement of intravenous injection. Meanwhile, the encapsulation efficiency and drug loading of PCL–PEG–PCL were about 99% and 20%, respectively. It implied that PCL–PEG–PCL nanoparticle was suitable for honokiol delivery, which might be due to the good affinity between honokiol and PCL-PEG-PCL copolymer. At last, PCL–PEG–PCL nanoparticles encapsulated honokiol could also effectively inhibit both cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) human ovarian cancer cells proliferation *in vitro*. It was indicated that the obtained honokiol loaded PCL–PEG–PCL nanoparticles might have potential application in ovarian cancer therapy. Previously, honokiol was loaded into F127 ($PEO_{100}PPO_{70}PEO_{100}$) micelles to overcome its poor water solubility (Gou et al., 2008d). But F127 is not biodegradable, though F127 has good biocompatibility. Meanwhile, honokiol was also incorporated into PCL–PEG–PCL nanoparticle by emulsion solvent evaporation method using F127 as surfactant (Gou et al., 2008e). In that protocol, surfactant and violent mechanical stirring must be applied. Otherwise, liposomal honokiol was also developed by Jiang et al. (2008). Li et al. suggested that liposomal honokiol induces apoptosis and inhibits angiogenesis of ovarian tumor cells (Li et al., 2008). Luo et al. indicated that liposomal honokiol provided an effective approach to inhibit tumor growth in both cisplatin-sensitive and cisplatin-resistant human ovarian cancer with minimal side effects (Luo et al., 2008). Recently, Wei et al. prepared a novel honokiol formulation based on self-assembled PCL–PEG–PCL micelles without using any surfactants and organic solvents. But those micelles were thermo-sensitive which might result in the poor stability. Meanwhile, the drug loading was very low in that formulation (Wei et al., 2009). In this article, the prepared honokiol formulation based on PCL–PEG–PCL nanoparticles was different from previous honokiol formulations. This formulation might have the following advantages: (1) small particle size (80 nm), (2) monodisperse, (3) moderate preparation condition, (4) without surfactants and (5) high drug loading. The disadvantages of this formulation might be that organic solvent was used when nanoparticles were prepared, though the DMSO was low-toxic. In a word, the prepared honokiol loaded PCL–PEG–PCL nanoparticle was a novel honokiol formulation and might be an effective anticancer agent for human ovarian cancer.

5. Conclusion

Mono-dispersed blank or honokiol loaded PCL–PEG–PCL nanoparticles without any surfactants are successfully prepared by solvent diffusion method in a moderate condition. The PCL–PEG–PCL nanoparticle is low-toxic and suitable for honokiol delivery to overcome its poor solubility. The PCL–PEG–PCL nanoparticle encapsulated honokiol, as the same as free honokiol, can effectively inhibit both cisplatin-sensitive (A2780s) and -resistant (A2780cp) human ovarian cancer cells proliferation in a dose-dependent manner *in vitro*.

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