

# Preparation and characterization of monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) micelles for the solubilization and in vivo delivery of luteolin

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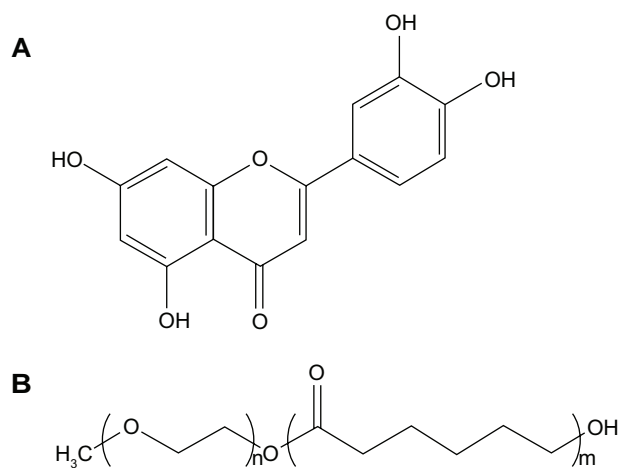
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**Abstract:** Luteolin (Lu) is one of the flavonoids with anticancer activity, but its poor water solubility limits its use clinically. In this work, we used monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles to encapsulate Lu by a self-assembly method, creating a water-soluble Lu/MPEG-PCL micelle. These micelles had a mean particle size of  $38.6 \pm 0.6$  nm (polydispersity index =  $0.16 \pm 0.02$ ), encapsulation efficiency of  $98.32\% \pm 1.12\%$ , and drug loading of  $3.93\% \pm 0.25\%$ . Lu/MPEG-PCL micelles could slowly release Lu in vitro. Encapsulation of Lu in MPEG-PCL micelles improved the half-life ( $t_{1/2}$ ;  $152.25 \pm 49.92$  versus [vs]  $7.16 \pm 1.23$  minutes,  $P = 0.007$ ), area under the curve (0–t) ( $2914.05 \pm 445.17$  vs  $502.65 \pm 140.12$  mg/L/minute,  $P = 0.001$ ), area under the curve (0– $\infty$ ) ( $2989.03 \pm 433.22$  vs  $503.81 \pm 141.41$  mg/L/minute,  $P = 0.001$ ), and peak concentration ( $92.70 \pm 11.61$  vs  $38.98 \pm 7.73$  mg/L,  $P = 0.003$ ) of Lu when the drug was intravenously administered at a dose of 30 mg/kg in rats. Also, Lu/MPEG-PCL micelles maintained the cytotoxicity of Lu on 4T1 breast cancer cells ( $IC_{50} = 6.4 \pm 2.30$   $\mu$ g/mL) and C-26 colon carcinoma cells ( $IC_{50} = 12.62 \pm 2.17$   $\mu$ g/mL) in vitro. These data suggested that encapsulation of Lu into MPEG-PCL micelles created an aqueous formulation of Lu with potential anticancer effect.

**Keywords:** luteolin, micelle, MPEG-PCL, cancer therapy

## Introduction

Cancer is the leading cause of death in developed countries and the second-leading cause of death in developing countries.<sup>1</sup> As the incidence of cancer is increasing, considerable attention is being paid to the prevention and therapy of cancer. Nonetheless, there are still many challenges in cancer therapy, and developing novel anticancer drugs is of interest. Natural products derived from plants play an important role in the health care of many cultures, both ancient and modern.<sup>2–4</sup> Presently, over 100 new natural products are in clinical development, in particular anticancer and anti-infective agents.<sup>4</sup> Natural compounds with antitumor activity and limited toxicity are getting more and more attention in medical research. Luteolin (Lu; 3',4',5',7'-tetrahydroxyflavone, shown in Figure 1A) belongs to a group of natural compounds named flavonoids that are found widely in the plant kingdom.<sup>5</sup> As with other flavonoids, Lu is often found in glycosylated form in many types of plants, including fruits, vegetables, and medicinal herbs (eg, green pepper, celery, broccoli, and parsley), and especially high amounts are found in peanut hulls and in *Reseda luteola* L.<sup>6</sup> In traditional Chinese medicine, plants rich in Lu have been used for treating various diseases, such as hypertension, inflammatory disorders, and cancer.<sup>5</sup> Recent studies have indicated that Lu possesses multiple biological effects,



**Figure 1** (A) Molecular structure of luteolin. (B) Molecular structure of monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) copolymer.

such as anti-inflammation, antiallergy, and anticancer, chiefly due to its antioxidant and free radical-scavenging capacities.<sup>6–8</sup> It was suggested that the number of hydroxyl groups in the structure of flavonoids is directly related to their free radical-scavenging activity.<sup>9</sup> With four hydroxyl groups, Lu is highly potent in scavenging free radicals and antioxidants. The anticancer effect of Lu also is associated with inducing apoptosis, which involves redox regulation, DNA damage, and protein kinases in inhibiting the proliferation of cancer cells and suppressing metastasis and angiogenesis.<sup>6,10–17</sup> Moreover, Lu has cytotoxicity in cancer cells or immortalized cells, but not in normal cells, meaning that it has fewer side effects when used in treating cancer.<sup>8,12,18</sup> Yet Lu has some drawbacks, such as poor water solubility ( $<2 \times 10^{-2} \mu\text{mol/mL}$ ),<sup>19</sup> low oral absorption,<sup>20</sup> and bioavailability (30.4% in rats),<sup>21</sup> which limit its clinical application. Therefore, it is of interest to promote an injectable aqueous formulation for Lu.

Nanotechnology has the potential to overcome the poor water solubility of lipophilic drugs.<sup>22</sup> Encapsulation of hydrophobic drugs into nanoparticles is a promising approach to make the drug intravenously injectable. The amphiphilic nanoparticles that comprise a hydrophobic core and a hydrophilic shell are excellent candidates for carrying hydrophobic drugs. Poly(ε-caprolactone)/poly(ethylene glycol) (PCL/PEG) can self-assemble into nanoparticles with core-shell structure: a hydrophobic PCL core and a hydrophilic PEG shell.<sup>23</sup> While encapsulated with the hydrophobic drug, the hydrophobic PCL segment combined with the drug forms the core and the hydrophilic PEG forms the shell of nanoparticles, making the drug intravenously injectable.<sup>24</sup> Also, encapsulated with hydrophobic drugs,

PCL/PEG copolymers could enhance stability and systemic circulation  $t_{1/2}$  of drugs and release drugs at a sustained rate in the optimal range of drug concentration.<sup>23,24</sup> Moreover, PCL/PEG copolymers are biodegradable, biocompatible, and easy to produce, showing promising applications in drug-delivery systems.<sup>25,26</sup>

Monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL) is a diblock PCL/PEG copolymer, and its molecular structure is presented in Figure 1B. Recently, MPEG-PCL micelles were used to deliver hydrophobic drugs (such as honokiol and curcumin), with the goal of creating novel aqueous formulations for these drugs.<sup>24,26</sup> In this study, in an attempt to promote an aqueous formulation for Lu, we prepared Lu-encapsulated MPEG-PCL micelles. Our results indicated that encapsulation of Lu in MPEG-PCL micelles rendered Lu completely dispersible in water. Also, Lu/MPEG-PCL micelles were able to slowly release Lu and efficiently inhibit the growth of cancer cells in vitro. These data suggested that Lu/MPEG-PCL micelles may be a novel nanoformulation of Lu with promising application in cancer therapy.

## Materials and methods

### Materials

Lu was purchased from Skyherb (Huangzhong, People's Republic of China). MPEG-PCL diblock copolymer with a designed molecular weight of 4000 was prepared according to our previous report.<sup>26</sup> Sprague Dawley rats (weight of  $200 \pm 20$  g) were purchased from the Laboratory Animal Center of Sichuan University (Chengdu, People's Republic of China). The 4T1 breast cancer cells and C-26 colon carcinoma cells were purchased from the ATCC (American Type Culture Collection, Rockville, MD, USA). All studies involving rats were approved by the institute's animal care and use committee.

### Preparation of Lu/MPEG-PCL micelles

Lu/MPEG-PCL micelles were prepared by a self-assembled method. Briefly, Lu and MPEG-PCL diblock copolymer mixture were codissolved in 5 mL of acetone first; next, the solution was evaporated in a rotator evaporator. Then, 5 mL of normal saline was added, followed by shaking gently in a 55°C water bath. In this process, MPEG-PCL self-assembled into core-shell-structured micelles with core-encapsulated Lu. The Lu-loaded MPEG-PCL micelle solution was centrifuged at  $13 \times 10^3$  rpm for 2 minutes to remove the insoluble drugs. Finally, the prepared Lu/MPEG-PCL micelles were lyophilized and stored at 4°C.

## Characterization of Lu/MPEG-PCL micelles

The particle size and zeta potential of Lu/MPEG-PCL micelles were obtained from three repeat measurements by a dynamic laser-diffraction particle-size detector and a Malvern Zeta analyzer (Nano-ZS; Malvern Instruments, Malvern, UK), respectively. During the measuring process, the temperature was kept at 25°C.

Morphology of the Lu/MPEG-PCL micelles was observed under a transmission electron microscope (H-6009IV; Hitachi, Tokyo, Japan): micelles were dissolved with distilled water and placed on a copper grid covered with nitrocellulose. Samples were negatively stained with phosphotungstic acid and dried at room temperature.

The concentration of Lu was determined by high-performance liquid chromatography (HPLC) (Alliance 2695; Waters, Milford, MA, USA). Chromatographic separations were performed on a reversed-phase C18 column (250 × 4.6 mm, 5 μm, SunFire Analysis column; Waters), with a mobile phase composed of methanol and 0.2% phosphoric acid aqueous solution (58:42, v/v) at a flow rate of 1.0 mL/minute (injected volume, 20 μL). The detection wavelength and the column temperature were set at 360 nm and 30°C, respectively. The amount of Lu was estimated from standard curves obtained by analysis of various doses of a standard substance.

To measure drug loading (DL) and encapsulation efficiency (EE) of Lu/MPEG-PCL micelles, 0.1 mL of Lu/MPEG-PCL micelle solution was placed in a preweighed Eppendorf tube and lyophilized to constant weight. Afterwards, the precipitate was dissolved in 0.1 mL acetone and was diluted by methanol to a total volume of 4 mL. The amount of Lu in the solution was assayed using HPLC. Finally, the DL and EE of Lu-loaded MPEG-PCL micelles were calculated according to equations (1) and (2):

$$DL = \frac{\text{Drug}}{\text{Polymer} + \text{Drug}} \times 100\% \quad (1)$$

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

## In vitro release study

To determine the release profile of Lu from Lu/MPEG-PCL micelles, 1 mL of Lu/MPEG-PCL micelle water solution and Lu in dimethyl sulfoxide solution (as control) at a concentration of 1 mg/mL was placed in a dialysis bag with a molecular weight cutoff of 7 kDa. These dialysis bags were incubated

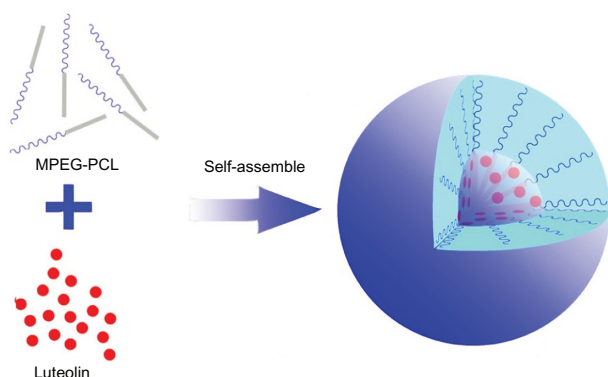
in 30 mL of phosphate-buffered saline (pH 7.4) containing Tween-80 (0.5%, v/v) at 37°C with gentle shaking. At predetermined time points, the incubation medium was changed for fresh incubation medium. At each data point, the incubation medium from these two samples was collected separately, the amount of released drug in them was quantified by HPLC, and the accumulated release profile with time was demonstrated. This study was repeated three times, and the results were expressed as mean values ± standard deviation.

## In vivo pharmacokinetics study

Rats were subjected to jugular venous cannulation 24 hours prior to assays. Under chloral hydrate anesthesia, 2 cm of the cannula was introduced into the jugular vein toward the heart. The inside of the implanted cannula remained permanently filled with heparin solution, and the free end of the cannula was closed with a plug. These rats were fasted overnight prior to drug administration, and were divided into two groups (three rats in each group): an Lu-treatment group and an Lu/MPEG-PCL micelle-treatment group. Free Lu was dissolved in Cremophor EL (polyoxyl 35 castor oil) and alcohol (1:1, v/v) solution, followed by dilution with normal saline. Lu/MPEG-PCL micelles were dissolved in normal saline, followed by intravenous administration of 30 mg/kg of free Lu or micelle-encapsulated Lu (Lu/MPEG-PCL micelles). The blood was collected from the jugular venous cannula at different time intervals. Plasma was separated, and Lu was extracted from plasma with acetonitrile; supernatant fluid was collected and evaporated to dryness by nitrogen-blowing. The dry residues were dissolved in methanol for HPLC analysis. Peak concentration ( $C_{\max}$ ) and time to reach  $C_{\max}$  ( $T_{\max}$ ) values were recorded directly from the measured data. Then,  $t_{1/2}$ , area under the curve ( $AUC$ )<sub>0-t</sub>, and  $AUC$ <sub>0-∞</sub> values were calculated. Drug and Statistics (DAS) software (version 2.1.1; Mathematical Pharmacology Professional Committee of China), was used for pharmacokinetic analysis.

## Cytotoxicity study of Lu/MPEG-PCL micelles in vitro

The cytotoxicity of free Lu or MPEG-PCL-encapsulated Lu to tumor cell lines was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The 4T1 (breast) and C26 (colon) tumor cell lines were maintained in Roswell Park Memorial Institute 1640 culture medium with 10% fetal bovine serum and incubated at 37°C in a humidified atmosphere (95% air and 5% CO<sub>2</sub>). The 4T1 or C26 cells were plated at a density of 5 × 10<sup>3</sup> cells per well in 100 μL of culture medium in 96-well plates, and were incubated at



**Figure 2** Preparation scheme of luteolin/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles by a self-assembly method. Luteolin and MPEG-PCL were codissolved in acetone followed by evaporation of the acetone. Then, normal saline was added making the self-assembly of the core-shell-structured micelles with core-encapsulated luteolin.

37°C for 24 hours. Cells were then treated for 48 hours with different concentrations of free Lu or micelle-encapsulated Lu or blank MPEG-PCL micelles. Control cultures were treated with 0.1% dimethyl sulfoxide; this concentration was not found to affect cell growth. The effect of Lu on cell viability was determined using MTT, a colorimetric assay with the reduction of MTT as the assessable end point. Data were shown as proportional to cell viability (%) by comparing the treated group with the untreated cells.

## Statistical analysis

Data are expressed as mean values  $\pm$  standard deviation. Statistical analysis was performed with unpaired Student's *t*-tests. *P*-values less than 0.05 were considered to be statistically significant.

## Results

### Preparation and characterization of Lu/MPEG-PCL micelles

Lu/MPEG-PCL micelles were prepared by a self-assembly method; a schematic diagram is shown in Figure 2. Altered ratios of Lu and MPEG-PCL diblock copolymer were

codissolved in 5 mL of acetone. Then the acetone was evaporated under vacuum in a rotary evaporator, leaving a layer of pale-yellow transparent membranous substances adhering to the inside wall of the glass container. Then, 5 mL of normal saline was added, followed by shaking in a 55°C water bath until the membranous substances were completely dissolved. In this process, the hydrophobic Lu was encapsulated into the core of the MPEG-PCL micelles, creating Lu/MPEG-PCL micelles. To optimize the process parameters, different Lu/MPEG-PCL mass ratios in the feed were studied in our work, and results are presented in Table 1. With the increase in Lu/micelle ratio, drug loading increased, but encapsulation efficiency decreased; also, particle size and polydispersity index (PDI) increased, but the stability of the Lu/MPEG-PCL micelles decreased. When the Lu/MPEG-PCL mass ratio was  $\geq 5/95$ , the obtained Lu/MPEG-PCL micelles were not stable in water (micelles tended to aggregate, forming aggregates); thus, the Lu/MPEG-PCL mass ratio at 4/96 was chosen in our study.

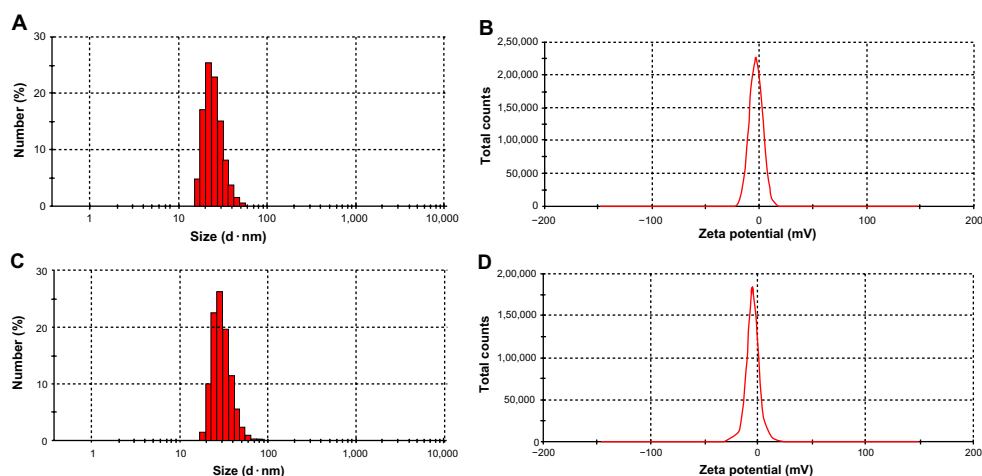
Sample III was characterized in detail and used for future applications. Drug loading and encapsulation efficiency of Lu/MPEG-PCL micelles were  $3.93\% \pm 0.25\%$  and  $98.32\% \pm 1.12\%$ , respectively. Moreover, these Lu/MPEG-PCL micelles had a median particle size of  $38.6 \pm 0.6$  nm (PDI =  $0.16 \pm 0.02$  nm) and a mean slight negative surface charge of  $-3.54 \pm 0.32$  mV. The particle-size distribution spectrum of freshly prepared Lu/MPEG-PCL micelles is presented in Figure 3A, showing that Lu/MPEG-PCL micelles had a narrow particle-size distribution. The zeta-potential distribution spectrum of Lu/MPEG-PCL micelles indicated that Lu/MPEG-PCL micelles had a slight negative surface charge (shown in Figure 3B).

After Lu/MPEG-PCL micelles were freeze-dried, the resolubility of these lyophilized Lu/MPEG-PCL micelles was examined. As shown in Figure 3C, these redissolved micelles had a mean particle size of  $45.8 \pm 2.2$  nm (PDI =  $0.15 \pm 0.04$ ). These redissolved micelles had a mean slight negative surface charge of  $-4.75 \pm 0.68$  mV, and the zeta-potential distribution

**Table 1** Properties of luteolin-loaded monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles

Sample	Luteolin/MPEG-PCL mass ratio	DL (%)	EE (%)	Mean size (nm)	PDI	Stability
I	0/100	0	0	$23.4 \pm 0.2$	$0.10 \pm 0.01$	Yes
II	2/98	$1.99 \pm 0.01$	$99.50 \pm 0.50$	$24.5 \pm 0.3$	$0.12 \pm 0.01$	Yes
III	4/96	$3.93 \pm 0.25$	$98.32 \pm 1.12$	$38.6 \pm 0.6$	$0.16 \pm 0.02$	Yes
IV	5/95	$4.72 \pm 0.07$	$94.49 \pm 1.31$	$43.7 \pm 0.1$	$0.18 \pm 0.02$	No
V	6/94	$5.55 \pm 0.05$	$92.56 \pm 0.84$	$46.6 \pm 0.2$	$0.23 \pm 0.04$	No

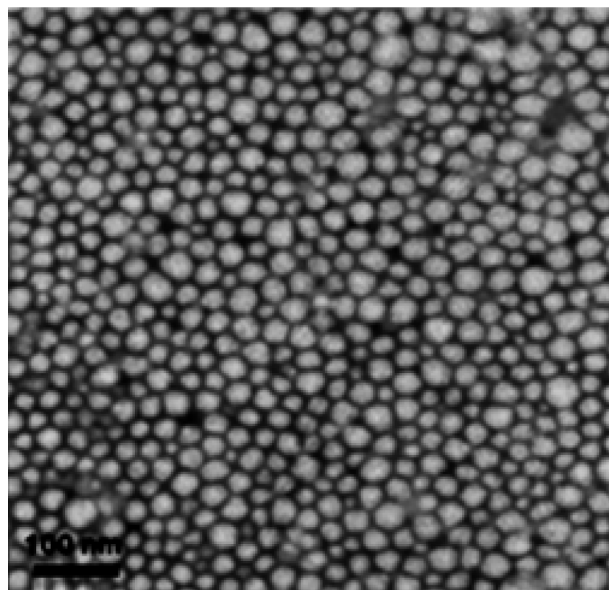
**Abbreviations:** EE, encapsulation efficiency; DL, drug loading; PDI, polydispersity index; nm, nanometer.



**Figure 3 (A–D)** Particle-size distribution and zeta-potential distribution spectra of luteolin (Lu)/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles. **(A and B)** Particle-size distribution spectrum and zeta-potential distribution spectrum of freshly prepared Lu/MPEG-PCL micelles, respectively; **(C and D)** particle-size distribution spectrum and zeta-potential distribution spectrum of redissolved Lu/MPEG-PCL micelles after freeze-drying, respectively. Each test was repeated three times.

spectrum is shown in Figure 3D. The morphology of the Lu/MPEG-PCL micelles, determined by transmission electron microscopy, is shown in Figure 4, revealing that Lu/MPEG-PCL micelles were uniform and spherical, with a diameter of  $\sim 30$  nm.

The appearance of the Lu/MPEG-PCL micelle aqueous solution is shown in Figure 5. Pure Lu could not be dissolved in pure water (Figure 5D), as confirmed by the observation of turbid oyster white slurry. However, Lu/MPEG-PCL micelle solution loaded with an equivalent quantity of Lu was transparent (Figure 5B), indicating the drug-loaded micelles

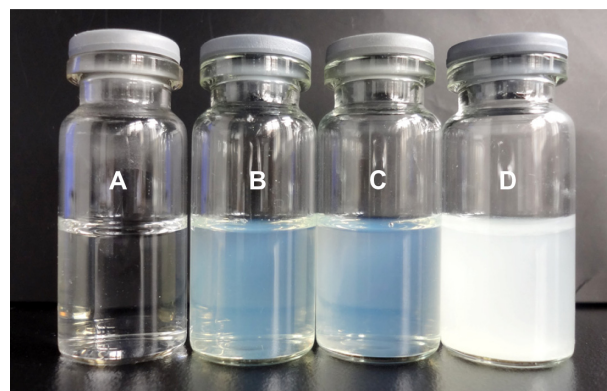


**Figure 4** Transmission electron microscopy image of luteolin/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) micelles.

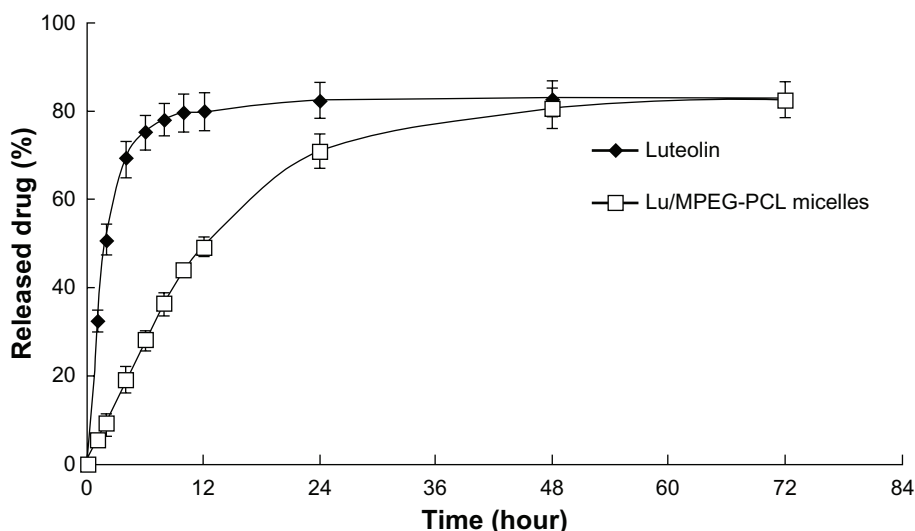
were fully dispersible in water. Freeze-dried Lu/MPEG-PCL micelles (Figure 5C) were also fully dispersible in water. Thus, we can draw a conclusion that encapsulation of Lu in MPEG-PCL micelles renders Lu completely dispersible in aqueous media, making Lu intravenously injectable.

### In vitro release profile

The in vitro release behavior of MPEG-PCL micelle-encapsulated Lu and free Lu in phosphate-buffered saline (pH 7.4) at  $37^\circ\text{C}$  was studied using a dialysis method, and the results are shown in Figure 6. Free Lu was observed to be rapidly released and reached its peak of 82.8% of the total in the first 12 hours. In comparison, MPEG-PCL micelle-encapsulated Lu showed a two-phase release profile. A relatively rapid release of MPEG-PCL micelle-encapsulated Lu in the first



**Figure 5 (A–D)** Luteolin (Lu)/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles render Lu completely dispersible in aqueous media. **(A)** Water as control; **(B)** freshly prepared Lu/MPEG-PCL micelle solution; **(C)** redissolved Lu/MPEG-PCL micelles after freeze-drying; **(D)** pure Lu in water.



**Figure 6** In vitro release profile of luteolin (Lu)/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles. The in vitro release profiles were examined using a dialysis method. Lu could be slowly released from micelles in vitro. The test was repeated three times.

phase was observed in the first 12 hours (49.3%), followed by a sustained and slower release phase. About 82.5% of Lu was released from Lu-encapsulated MPEG-PCL micelles in 72 hours. The results indicated that Lu could be slowly released from micelles in a sustained manner in vitro. The sustained release of Lu from MPEG-PCL micelles might be due to two reasons: (1) the diffusion of Lu from the micelles to the medium, and (2) degradation and hydrolysis of micelle-induced release of Lu to the medium.

### In vivo pharmacokinetics analysis

To confirm whether Lu/MPEG-PCL micelles can improve the pharmacokinetics of Lu in vivo, the pharmacokinetics of free Lu and Lu/MPEG-PCL micelles was studied in rats. Rats were intravenously administered with Lu or Lu/MPEG-PCL micelles (Lu 30 mg/kg). Blood was collected at different time intervals. Results of pharmacokinetics are shown in Table 2 and Figure 7 and were analyzed by DAS software. For MPEG-PCL micelle-encapsulated Lu,  $T_{max}$ ,  $t_{1/2}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , and  $C_{max}$  were 5 minutes,  $152.25 \pm 49.92$  minutes,  $2914.05 \pm 445.17$  mg/L/minute,  $2989.03 \pm 433.22$  mg/L/minute, and  $92.70 \pm 11.61$  mg/L, respectively. For pure Lu,  $T_{max}$ ,  $t_{1/2}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , and  $C_{max}$  were 5 minutes,  $7.16 \pm 1.23$  minutes,  $502.65 \pm 140.12$  mg/L/minute,

$503.81 \pm 141.41$  mg/L/minute, and  $38.98 \pm 7.73$  mg/L, respectively. Thus, it was suggested that encapsulation of Lu in MPEG-PCL micelles improved  $t_{1/2}$  ( $P = 0.007$ , Lu/MPEG-PCL versus [vs] Lu),  $AUC_{0-t}$  ( $P = 0.001$ , Lu/MPEG-PCL vs Lu),  $AUC_{0-\infty}$  ( $P = 0.001$ , Lu/MPEG-PCL vs Lu), and  $C_{max}$  ( $P = 0.003$ , Lu/MPEG-PCL vs Lu) of Lu in vivo.

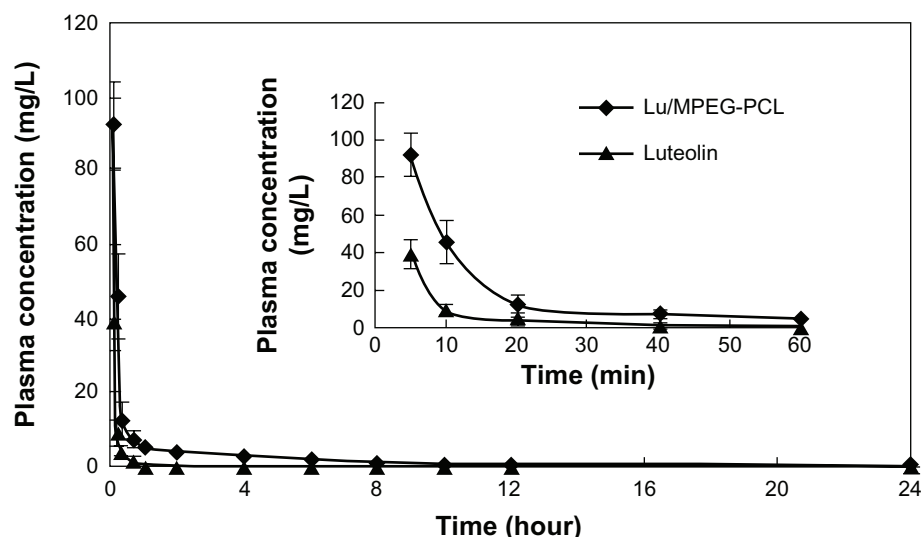
### In vitro cytotoxicity of Lu/MPEG-PCL micelles

The cytotoxicity of Lu/MPEG-PCL micelles was compared with that of pure Lu on 4T1 breast cancer cells and C26 colon carcinoma cells in vitro. After cancer cells were treated with micelle-encapsulated Lu, pure Lu or blank MPEG-PCL micelles for 48 hours, cell viability was measured by MTT assay. As shown in Figure 8, the blank MPEG-PCL micelles did not show any cytotoxicity activity in 4T1 cancer cells or C26 cancer cells, while both pure Lu and Lu/MPEG-PCL micelles showed significant cytotoxic activity in both of these two cell lines. Even the cytotoxicity of MPEG-PCL micelle-encapsulated Lu was stronger than that of free Lu. The half-maximal inhibitory concentrations for free Lu and MPEG-PCL micelle-encapsulated Lu on 4T1 cells were  $10.21 \pm 1.64$   $\mu$ g/mL and  $6.4 \pm 2.30$   $\mu$ g/mL, respectively, and those in C26 cells were  $13.06 \pm 1.84$   $\mu$ g/mL and

**Table 2** Pharmacokinetic parameters for both formulations

Groups	$T_{max}$ minutes	$t_{1/2}$ minutes	$AUC_{(0-t)}$ mg/L/minute	$AUC_{(0-\infty)}$ mg/L/minute	$C_{max}$ mg/L
Luteolin	5	$7.16 \pm 1.23$	$502.65 \pm 140.12$	$503.81 \pm 141.41$	$38.98 \pm 7.73$
Lu/MPEG-PCL	5	$152.25 \pm 49.92$	$2914.05 \pm 445.17$	$2989.03 \pm 433.22$	$92.70 \pm 11.61$
<i>P</i>	–	0.007	0.001	0.001	0.003

**Abbreviations:**  $T_{max}$ , time to reach  $C_{max}$ ;  $t_{1/2}$ , half-life; AUC, area under the curve;  $C_{max}$ , peak concentration; MPEG-PCL, monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone).



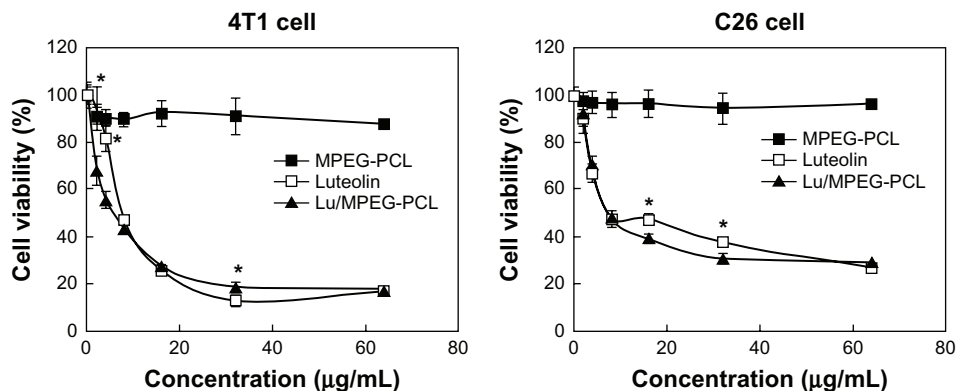
**Figure 7** In vivo pharmacokinetics assays of luteolin (Lu)/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles. Encapsulation of luteolin in MPEG-PCL micelles improved the pharmacokinetics of Lu. The test was repeated three times.

$12.62 \pm 2.17 \mu\text{g/mL}$  ( $P > 0.05$ ), respectively. This result indicated that MPEG-PCL micelle encapsulated-Lu as well as free Lu still had anticancer activity.

## Discussion

To overcome the poor water-solubility of Lu, we encapsulated Lu into MPEG-PCL micelles by a self-assembly method in this study, producing Lu/MPEG-PCL micelles. The Lu/MPEG-PCL micelles were about 40 nm in diameter, rendering Lu completely dispersible in water (Figure 5). Also, Lu/MPEG-PCL micelles showed drug loading of  $3.93\% \pm 0.25\%$  and a high encapsulation efficiency of  $98.32\% \pm 1.12\%$ , making this an aqueous formulation of Lu. In addition, the Lu/MPEG-PCL micelles had a slight negative surface charge of  $-3.54 \pm 0.32 \text{ mV}$ , which increased the circulation time of

the drug. Surface charge is important in determining whether the nanoparticles will cluster in blood flow or will adhere to or interact with oppositely charged cell membrane.<sup>27</sup> The plasma and blood cells always had a negative charge; nanoparticles with slight negative surface charge may minimize nonspecific interaction with these components through electrostatic interactions.<sup>28–30</sup> The Lu/MPEG-PCL micelles were able to slowly release Lu (Figure 6), indicating that Lu-encapsulated MPEG-PCL micelles may enhance systemic circulation  $t_{1/2}$  of Lu in vivo. The sustained release of Lu from MPEG-PCL micelles might be due to the diffusion of Lu from micelles and the degradation or hydrolysis of micelles. Pharmacokinetics in rats was studied, and the results (Figure 7) proved that Lu/MPEG-PCL micelles enhance systemic circulation  $t_{1/2}$  of Lu in vivo. Meanwhile, the Lu/MPEG-PCL micelles



**Figure 8** Cytotoxicity of luteolin (Lu)/monomethoxy poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (MPEG-PCL) micelles on cancer cells in vitro. As well as free Lu, Lu/MPEG-PCL micelles could efficiently inhibit the viability of both 4T1 breast cancer cells and C26 colon cancer cells, while blank MPEG-PCL micelles had little cytotoxicity on these cells. The test was repeated three times.

**Notes:** \*significant difference between luteolin and Lu/MPEG-PCL.

maintained the cytotoxicity of Lu, which showed in an in vitro cytotoxicity study in 4T1 and C26 cells (Figure 8). The  $IC_{50}$  values for pure Lu and MPEG-PCL micelle-encapsulated Lu in 4T1 cells were  $10.21 \pm 1.64 \mu\text{g/mL}$  and  $6.4 \pm 2.30 \mu\text{g/mL}$ , respectively, and those in C26 cells were  $13.06 \pm 1.84 \mu\text{g/mL}$  and  $12.62 \pm 2.17 \mu\text{g/mL}$ , respectively.

Lu is a promising agent for anticancer therapy. But Lu is a lipophilic drug and insoluble in water, which limits its clinical application. Encapsulation by nanoparticles appears to be a promising approach to overcome the hydrophobicity of drugs. There are many encapsulation methods, such as spray-drying, solvent removal, etc, to encapsulate Lu into micelles or liposomes.<sup>31</sup> Also, inclusion complexation with cyclodextrin could enhance the water solubility of Lu.<sup>32,33</sup> In this work, we used MPEG-PCL micelles to encapsulate Lu. In the preparation process, Lu and MPEG-PCL diblock copolymer mixture was first dissolved in acetone solution, followed by evaporating the organic solvent. Then, the amphiphilic MPEG-PCL copolymers self-assembled into supramolecular arrangements possessing a hydrophobic inner core and a hydrophilic shell in water, and Lu self-assembled into the hydrophobic core of the micelles because of its hydrophobicity. The preparation procedure was simple and easy to scale up. These MPEG-PCL micelles are biodegradable, biocompatible, amphiphilic, stable in blood, nontoxic, nonimmunogenic, non-inflammatory, and small in size; this makes MPEG-PCL micelles an excellent candidate for drug-delivery systems.<sup>27</sup> Thus, MPEG-PCL micelle-encapsulated Lu might be an interesting formulation.

In summary, MPEG-PCL micelles were used to encapsulate Lu, creating Lu/MPEG-PCL micelles. The Lu/MPEG-PCL micelles improved the water solubility, enhanced circulation  $t_{1/2}$  in vivo, and maintained the cytotoxicity of Lu. Lu/MPEG-PCL micelles are an injectable aqueous formulation of Lu and may have potential application in cancer treatment.

## Conclusion

MPEG-PCL micelle-encapsulated Lu was prepared, with the goal of improving the water solubility of Lu. These Lu/MPEG-PCL micelles can slowly release Lu, enhance systemic circulation  $t_{1/2}$  of Lu in vivo, and inhibit the growth of cancer cells in vitro, showing promising application in cancer treatment.

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## Disclosure

The authors report no conflicts of interest in this work.

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