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Preparation and characterization of cationic curcumin nanoparticles for improvement of cellular uptake

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ABSTRACT

In the present paper, cationic nanoparticles of curcumin, chitosan and poly(ε-caprolactone) were developed by a simple nano-precipitation method. The developed curcumin loaded chitosan/poly(ε-caprolactone) (chitosan/PCL) nanoparticle showed almost spherical shape and its diameter was varied between 220 nm and 360 nm and zeta potential was varied between +30 mV and 0 mV as a function with pH value. The encapsulation of curcumin into nanoparticles was confirmed by fluorescence spectral analysis. *In vitro* release study showed the sustained release behavior of curcumin from nanoparticles during the period of 5 days study. *In vitro* cytotoxicity test revealed the drug concentration dependent on the cell viability against Hela cells and OCM-1 cells after 48 h co-incubation. Furthermore, *in vitro* cell uptake study revealed that the cell uptake of curcumin was greatly enhanced by encapsulated curcumin into cationic chitosan/PCL nanoparticles. Therefore, the developed cationic chitosan/PCL nanoparticles might be a promising candidate for curcumin delivery to cancer cells.

1. Introduction

Cancer was the most distressing disease that enforces severe death worldwide. The common option for the treatment of cancer was chemotherapy, but that is often limited by unwanted toxic effects on normal tissues. This is because most anti-cancer agents are not distributed in the target tumor-bearing tissues specifically, which results in reduced therapeutic efficacy (Bilensoy et al., 2009; Gou et al., 2011). In the past three decades, polymeric nanoparticles have been used as a preferred nanoscale drug delivery vehicle for treatment of cancer due to their excellent endocytosis efficiency, passive tumor-targeting, high encapsulation efficiency, and delivery of a wide range of therapeutic agents (Brannon-Peppas & Blanchette, 2004; Brigger, Dubernet, & Couvreur, 2002; Peer et al., 2007).

Curcumin is a low molecular weight hydrophobic polyphenol derived from the rhizome of turmeric, which has been used for centuries as remedy for many diseases including neoplastic, neurological, cardiovascular, pulmonary and etc. (Aggarwal & Harikumar, 2009; Kunnumakkara, Anand, & Aggarwal, 2008; Lin, Lee, Chiu, & Hung, 2011). Recently, researchers found that curcumin has a diverse range of molecular targets such as growth factors, transcription factors and their receptors, cytokines enzymes and genes (Glienke, Maute, Wicht, & Bergmann, 2009; Lin et al., 2009). Curcumin could inhibit nuclear factor (NF)-κB activity in human pancreatic cells and decrease the expression of NF-κB regulated gene products, including cyclooxygenase (COX)-2, prostaglandin E2, and IL-8 (Jutooru, Chadalapaka, Lei, & Safe, 2010; Lin et al., 2007). Furthermore, the lower systemic toxicity at high dose (8 g/day in human clinical trials) associated with the sensitive to the cancer cells suggests the possible application of curcumin in cancer therapy (Bar-Sela, Epelbaum, & Schaffer, 2010; Maheshwari, Singh, Gaddipati, & Srimal, 2006). Despite there are favorable properties of curcumin, its extremely low solubility in water, poor bioavailability and degradation at alkaline pH have greatly limited its further *in vivo* applications (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Bar-Sela et al., 2010). Several formulations strategies including nanoparticles, liposomes, complexation with phospholipids and cyclodextrins, solid dispersions, nano-crystal and etc, have been developed to increase its aqueous solubility and bioavailability (Anand et al., 2010; Kim et al., 2011; Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009). Recently, curcumin was successfully encapsulated into polymeric nanoparticle of glycerol monooleate (GMO) and the data showed that nano-curcumin demonstrates more effective than native curcumin against

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different cancer cell lines assessed by *in vitro* cytotoxicity (Mohanty & Sahoo, 2010). Shaikh et al. (2009) reported that encapsulated curcumin into PLGA nanoparticles demonstrate at least 9-fold increase in oral bioavailability when compared to curcumin administration with piperine as absorption enhancer. These nanoparticles delivery systems based on synthetic polymers are currently gaining interest to augment the systemic bioavailability of curcumin. PCL as a non-toxic degradation product has been approved by Food and Drug Administration for various biomedical applications. There are numerous literatures on the synthesis of PCL micro/nanoparticles for drug delivery applications (Benoit, Baras, & Gillard, 1999; Neves et al., 2011; Shuai, Ai, Nasongkla, Kim, & Gao, 2004). However, PCL was extremely hydrophobic polymer that could not form nanoparticles spontaneously absence of any other emulsifier or stabilizer (Chawla & Amiji, 2002). Therefore, for prevention of aggregation of nanoparticles and for providing better stabilization to nanoparticles, coating/blending of large molecules such as polysaccharides or macromolecules was necessary. Chitosan as a cationic polysaccharide, is biodegradable, non-toxic and soft-tissue compatible, which has been widely used in the biomedical applications (Ilium, 1998; Rinaudo, 2006). As a cationic ligand, chitosan can facilitate the active transport of nanoparticles via absorptive-mediated transcytosis (AMT), and chitosan coated nanoparticles have been used as carrier for tumor target (Tahara, Sakai, Yamamoto, Takeuchi, & Kawashima, 2008; Zhu, Ma, Jia, Zhao, & Shen, 2009).

The aim of this paper was to develop curcumin loaded chitosan/PCL nanoparticles with appropriate surface modifications and well defined particle size that can improve the cell uptake of curcumin. As far as we know, this was the first time that the curcumin loaded chitosan/PCL nanoparticles were prepared by the simple nano-coprecipitation method from co-dissolved 90% acetic aqueous solution. Meanwhile, the physicochemical characteristics as well as *in vitro* anti-cancer efficiency of nanoparticles were investigated. Furthermore, *in vitro* cell uptake of curcumin/curcumin loaded chitosan/PCL nanoparticles was studied by fluorescence microscope and flow cytometry.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree 86%, viscosity 200 cP) was supplied by Sigma–Aldrich (USA). PCL (Mn = 14,000) was purchased from Sigma–Aldrich (USA). Curcumin was kindly provided by Aladdin Chemistry Co. Ltd. (China). Acetic acid was purchased from Dingsheng chemistry Co. Ltd. (China). All other chemicals used in this paper were analytical grade. Ultrapure water from Milli-Q water system was used to prepare the aqueous solutions.

2.2. Synthesis of curcumin loaded chitosan/PCL nanoparticles

In this paper, curcumin loaded chitosan/PCL nanoparticles were synthesized by a simple nano-coprecipitation method. Briefly, 0.1 g of curcumin, 0.05 g of chitosan and 0.95 g of PCL co-polymer were co-dissolved into 5 ml of 90% acetic acid solution to form a homogeneous solution under continuous stirring, and then added dropwise into 50 ml of distilled water solution to obtain the curcumin loaded chitosan/PCL nanoparticles. Finally, the obtained curcumin loaded chitosan/PCL nanoparticles were centrifuged at 13,000 rpm/min for 30 min, discarding the supernatant, and re-suspended with 10 ml of distilled water solution for the further characterization and applications.

2.3. Characterization

2.3.1. Particle size distribution and zeta potential measurement

Particle size and size distribution of nanoparticles as a function with the pH values (pH = 4.5, 5.5, 5.75, 6.8 and 10.5, respectively) were measured by laser diffraction (Zeta Plus-Zeta Potential Analyzer, Brookhaven Instruments Corporation, USA) after appropriate dilution of the nanoparticles with the corresponding medium (pH = 4.5, 5.5, 5.75, 6.8 and 10.5). Particle size distribution is reported as a polydispersity index (PDI). The temperature was maintained at 25 °C during the measurement. These measurements were performed in triplicates with independent particle batches. Zeta potential of nanoparticle was detected by a zeta potential analyzer (Zeta Plus-Zeta Potential Analyzer, Brookhaven Instruments Corporation, USA).

2.3.2. Transmission electron microscopy (TEM) observation

The morphology of obtained curcumin loaded chitosan/PCL nanoparticles was observed with a transmission electron microscopy (TEM) (H-6009IV, Hitachi, Japan); sample was diluted with distilled water solution and placed on a copper grid covered with nitrocellulose. The sample was negatively stained with 0.5% phosphotungstic acid and dried at room temperature before the observation.

2.3.3. Atomic force microscope (AFM) observation

The shape of obtained curcumin loaded chitosan/PCL nanoparticles was further characterized by atomic force microscope (AFM, SPA-400, Seiko Instruments Inc., Japan). A drop of nanoparticles suspension was placed on a mica followed by washed with distilled water solution three times to remove the unbound nanoparticles. After that, the sample was dried at room temperature and mounted on the microscope scanner for observation.

2.4. Encapsulation efficiency of nanoparticles

Drug loading efficiency and loading capacity of chitosan/PCL nanoparticles was measured by separation of nanoparticles from the 1 ml of aqueous nanoparticle suspension by centrifugation at 13,000 rpm/min for 20 min. The centrifuged nanoparticles pallet was lyophilized, weighted and recorded. The amount of free curcumin in the supernatant was determined by a High Performance Liquid Chromatography (HPLC, Aglilent 1200 series, USA) with a standard curve ($y = 128.634395 \times C - 3.1623221$; $R^2 = 0.99976$). HPLC analysis was performed on a reversed phase C18 column (4.6 mm × 150 mm, 5 μm, ZORBAX Eclipse XDB-C18) at room temperature. The mobile phase was composed of methanol and 0.3% acetic water solution (80:20, v/v); filtered through a 0.22 μm Millipore filter and degassed prior to use. The flow-rate was 1.0 ml/min and the eluent was detected by DAD detector at 420 nm. Drug loading efficiency and loading capacity were calculated respectively by the following formulas:

$$\text{Loading efficiency (LE, \%)} = \frac{\text{Total curcumin} - \text{free curcumin}}{\text{Total curcumin}} \times 100$$

$$\text{Loading capacity (LC, \%)} = \frac{\text{Total curcumin} - \text{free curcumin}}{\text{Weight of dried nanoparticles}} \times 100$$

2.5. Spectroscopic analysis

Encapsulation and binding of curcumin in hydrophobic core of the nanoparticles was further examined by fluorescence spectral analysis following the report of Mohanty, Acharya, Mohanty, Dilnawaz, and Sahoo (2010). The fluorescence spectra of both unmodified curcumin (dissolved into methanol solution) and

curcumin loaded chitosan/PCL nanoparticles were detected at concentration of 1.5 μg/ml. Curcumin was first quantified spectrophotometrically at 420 nm and fluorescence emission spectra were recorded from 450 to 800 nm with an excitation wavelength of 420 nm. The slit widths (modulation of magnitude and resolution of transmitted light) were standardized at 5 nm and 3.5 nm for excitation and emission wavelength, respectively.

2.6. In vitro release study

In vitro release behavior of curcumin from nanoparticles was performed by the dialysis method as previously reported with little modification (Kim et al., 2008). Briefly, 0.5 ml curcumin loaded chitosan/PCL nanoparticles of known curcumin drug concentration was placed into a dialysis bag (MWCO, 8000–14,000) followed by dialysis against 10 ml phosphate-buffered saline (PBS, pH = 7.4) solution at 37 °C. At specific time intervals, 1 ml of aliquot release medium was collected and the concentration of curcumin in medium was measured by HPLC method as described in Section 2.4. The resident release medium was re-placed with pre-warmed PBS solution for continuous study.

2.7. In vitro cytotoxicity study

In this paper, *in vitro* cytotoxicity of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles against Hela cells and OCM-1 cells was evaluated by MTT test as previously reported (Mohanty & Sahoo, 2010). Briefly, Hela cells and OCM-1 cells were separately seeded on 96-well plates with cell density of 5×10^3 cells/well and allowed to adhere for 24 h prior to the test. After that, a series of doses of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles were added and co-incubated at 37 °C for 48 h. Thereafter, 20 μl of MTT solution (5 mg/ml) was added to each well, and the cells were incubated at 37 °C for another 2 h in dark. Finally, the MTT solution was carefully withdrawn and 150 μl of DMSO was added in each well to dissolve the MTT formazan crystals. The absorbance was immediately measured at 570 nm by a microplate reader (Bio-Rad, Hercules, CA, USA). The cell viability (%) was related to the control wells containing untreated cells with fresh cell culture medium and was calculated according to the following formula: Cell viability (%) = (absorption test/absorption control) × 100. The values measured were expressed as mean value ± SD ($n = 6$).

2.8. In vitro cell uptake study

To examine the cellular uptake of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles, the fluorescence microscopic study and flow cytometry study were performed using Hela cells. Briefly, Hela cells were seeded on a 6-well plate with cell density at 1×10^5 cells/well and incubated for 24 h at 37 °C for attachment. After that, the attached cells were treated with a constant concentration (10 μg/ml) of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles for 3 h at 37 °C in a cell culture incubator. After incubation, the cell monolayers were rinsed three times with 1 ml PBS solution to eliminate curcumin or curcumin loaded chitosan/PCL nanoparticles that were not taken up by the cells. Two milliliter of fresh PBS solution was added to the plates and the cells were viewed and imaged by an inverted fluorescence microscope (at λ_{ex} 420 nm and λ_{em} 530 nm; IX71-F22FL/PH, Olympus). For the flow cytometry study, the cells after incubation with unmodified curcumin/curcumin loaded chitosan/PCL nanoparticles were transferred into polystyrene round-bottom tubes (5 ml) with 0.3 ml of PBS solution and maintained at 4 °C for subsequent flow cytometry analysis.

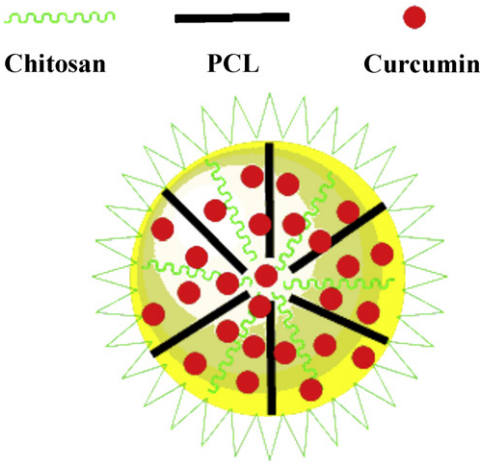

Fig. 1. Synthesis scheme of curcumin loaded chitosan/PCL nanoparticles.

2.9. Statistical analysis

Data was analyzed using the software program Origin Pro 7.5. Statistical comparison between unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles were determined by using one-way ANOVA using SPSS software ($p \leq 0.05$).

3. Results and discussion

3.1. Preparation and characterization of curcumin nanoparticles

In past three decades, numerous strategies for the manufacture of polymeric nanoparticles have been developed. Among these strategies, nano-coprecipitation was a simple and straightforward technique, which did not need any additional sonication or energy input (Wang et al., 2010). In this paper, the nano-coprecipitation from co-dissolved 90% acetic aqueous solution was the first time employed to develop curcumin loaded chitosan/PCL nanoparticles. With regard to the synthesis of nanoparticles, poly(vinyl alcohol) (PVA) was the most commonly used emulsifier. However, PVA as a toxic emulsifier was difficult to remove after emulsification, which was disadvantage for its further application (Feczko, Toth, & Gyenis, 2008). Recently, chitosan has been successfully applied in the formulation of PLGA, PCL and PEG-PCL nanoparticles, which exhibited a certain emulsifying effects, and the desired particle size and surface morphology (Tahara et al., 2008). Contrasted to PVA, chitosan was a cationic and safer emulsifier, and the chitosan molecules remaining on the surface of nanoparticles which allows the nanoparticles to be used for surface modifications and targeting applications (Yang et al., 2009). Generally, coating chitosan on to the nanoparticles was achieved by simple adsorption that was unstable and easy disaggregation at physiological condition. Herein, chitosan as an emulsifier was embedded into the nanoparticles (both on the surface and inner of nanoparticles) by a simple nano-coprecipitation method to encapsulate the curcumin intending for the improvement of cellular uptake. As depicted in Fig. 1, when dispersion of PCL, chitosan and curcumin co-dissolved 90% acetic aqueous solution to the distilled water solution, it allowed the hydrophobic PCL and curcumin as well as the amphiphilic chitosan to self-assemble into nanoparticles with a core–shell structure. Meanwhile, chitosan was also present on the surface of nanoparticles acting as the emulsifier and stabilizer, resulting in the positive charges of nanoparticles. Fig. 2A depicts the particle size and zeta potential of the curcumin loaded chitosan/PCL nanoparticles as a function with pH values. As presented in Fig. 2A, it clearly observed that the particle size and zeta potential of

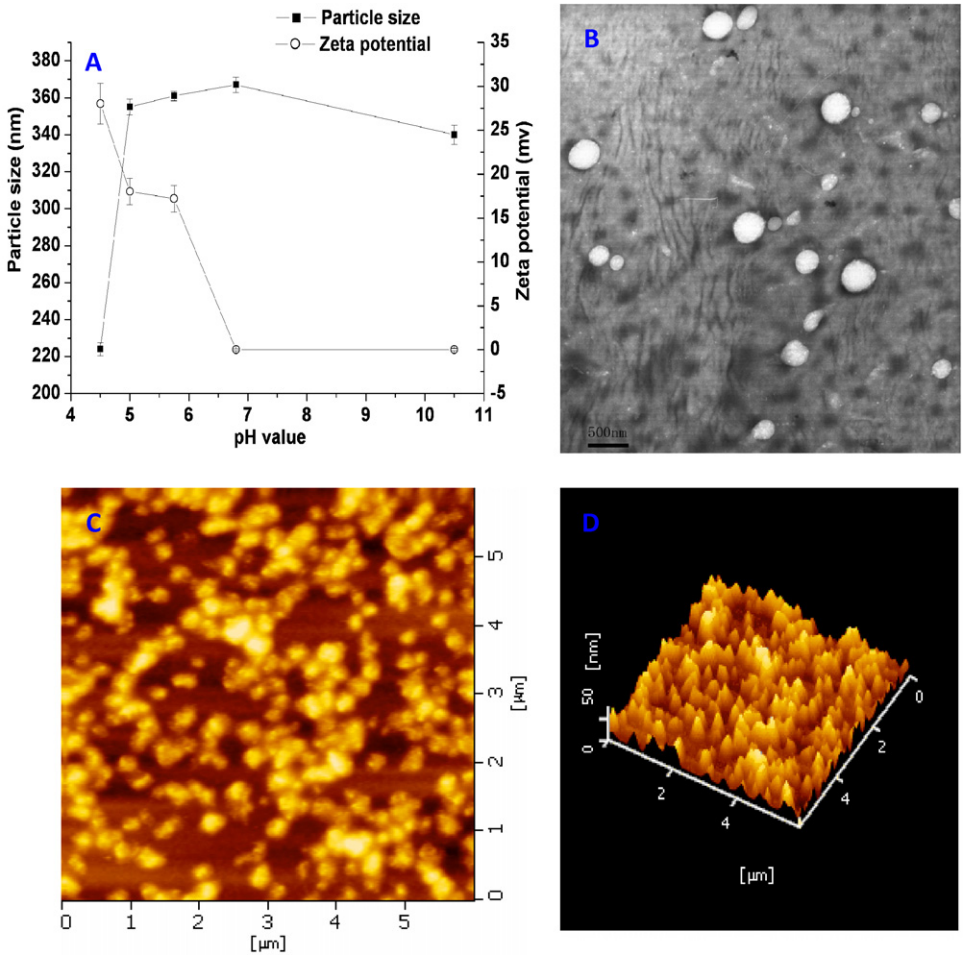

Fig. 2. (A) Particle size and zeta potential of curcumin loaded chitosan/PCL nanoparticles as the function with pH value. (B) TEM image of curcumin loaded chitosan/PCL nanoparticles at PBS solution (pH = 6.8). (C) Size distribution of curcumin loaded chitosan/PCL nanoparticles measured by AFM in PBS solution (pH = 6.8). (D) The three dimensional image of image C.

nanoparticles showed great dependence on the pH values. With the increase of pH value from 4.5 to 10.5, particle size of nanoparticles increased from 220 nm to 360 nm, yet the zeta potential of nanoparticles decreased from +30 mV to 0 mV. As placed in pH = 4.5, due to the protonation of amino groups in the chitosan skeleton, nanoparticles exhibited the cationic characterization (+30 mV). As the pH value was greater than pK_a (~6.2) of chitosan, the zeta potential of nanoparticles decreased to 0 mV due to the deprotonation of amino groups. For the particle size of nanoparticles, the aggregation of nanoparticles might contributed to the increase of particle size because of the decrease of electrostatic interactions between nanoparticles. Meanwhile, the morphology of obtained curcumin loaded chitosan/PCL nanoparticles at pH = 6.8 were observed by TEM and AFM, respectively. As depicted in Fig. 2B, spherical nanoparticle with well defined particle size (~250 nm) was observed by TEM, which was discrepancy with that of measurement of laser diffraction (~350 nm). This might be induce by the hydrodynamic diameter of nanoparticles was detected in aqueous solution, while the TEM observation revealed the morphology size of nanoparticles in solid state, which was in accordance with the previous report (Gou et al., 2011). From the AFM observation (Fig. 2C), it clearly revealed that the developed nanoparticles were almost spherical with size about 250 nm. However, in the 3-D graphics of nanoparticles (Fig. 2D), the height of nanoparticles was only about 70 nm, which was much smaller than that of plane size (~250 nm). This might be explained by the composition of nanoparticles (chitosan and PCL) were flexible polymers, resulting in the collapse of nanoparticles during the observation (Neves et al., 2011).

3.2. Drug loading efficiency and loading capacity

Curcumin was a water insoluble drug, which was suitable for encapsulation by nano-precipitation method to develop curcumin loaded chitosan/PCL nanoparticles. The encapsulation efficiency of nanoparticles was 70.9 ± 1.5% and loading capacity of nanoparticles was 4.1 ± 0.3%, which was used for the subsequent experiments.

3.3. Spectroscopic analysis

For further confirmation of encapsulation and binding of curcumin in the hydrophobic core of nanoparticles, the fluorimetric property of curcumin was detected, and the related results were presented in Fig. 3. As depicted in Fig. 3, it clearly observed that the fluorescence peak of unmodified curcumin in methanol solution was about 540 nm when excited at 420 nm, which was in accordance with the previous report of Mohanty et al. (2010). However, the fluorescence spectrum of curcumin loaded chitosan/PCL nanoparticles was shifted to the blue spectrum and showed a well-defined peak at 525 nm. This phenomenon might be explained by that the curcumin was encapsulated into the hydrophobic domain of the nanoparticles. Similarly, Sahu, Kasoju, and Bora (2008)

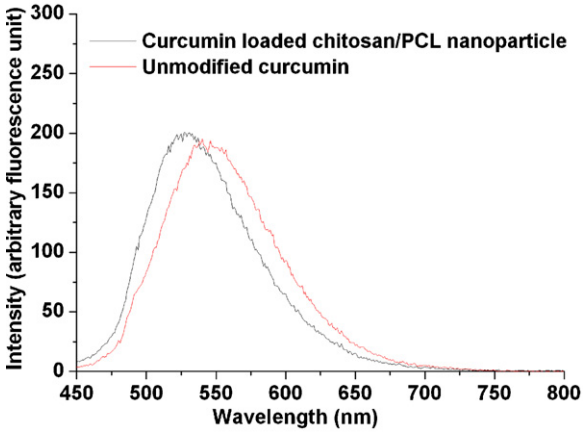

Fig. 3. The fluorescence emission spectra of methanolic solution of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticles when excited at 420 nm.

also reported that curcumin-encapsulated bovine casein micelle showed a shift in fluorescence spectra from 540 nm to 500 nm because of the binding of curcumin in the hydrophobic domain of the protein molecule in micelle. Therefore, we inferred that the blue shift of fluorescence spectrum in curcumin loaded chitosan/PCL nanoparticles when compared with that of unmodified curcumin was induced by the binding of curcumin to the hydrophobic domain of the nanoparticles through hydrophobic interactions.

3.4. In vitro release study

The release profile of curcumin from chitosan/PCL nanoparticles was studied *in vitro*, and the results were shown in Fig. 4. As presented in Fig. 4, we could find that a small initial burst release with only about 25% of the total drug was observed in the first 6 h, which might be attributed to diffusion of curcumin from chitosan/PCL nanoparticles. Since the curcumin diffuses out of the nanoparticles, the disaggregation or collapse of chitosan/PCL nanoparticles might be prevailed in the subsequent stage followed by sustained drug release of about 68% over 5 days. In order to preliminary investigate the release mechanism of curcumin from chitosan/PCL nanoparticles, we analyzed regression coefficients with three kinetic models (first order, zero order and Higuchi model). As a result, the Higuchi model was best fitted with release kinetic with regression coefficients (R^2) of 0.945, indicating that the diffusion of curcumin from nanoparticles was dominant at initial stage of release behavior. This

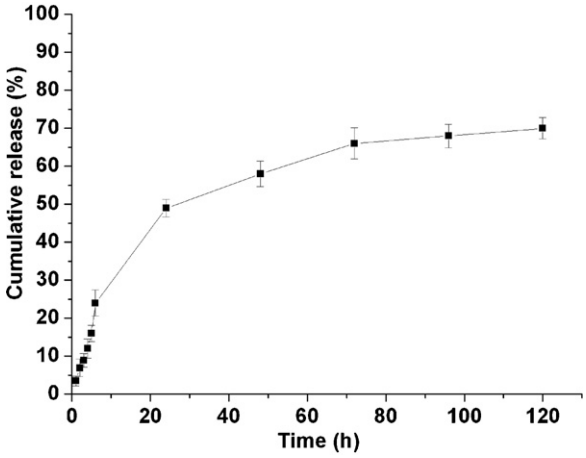

Fig. 4. In vitro release profile of curcumin from the chitosan/PCL nanoparticles in PBS solution (pH = 7.4) at 37 °C.

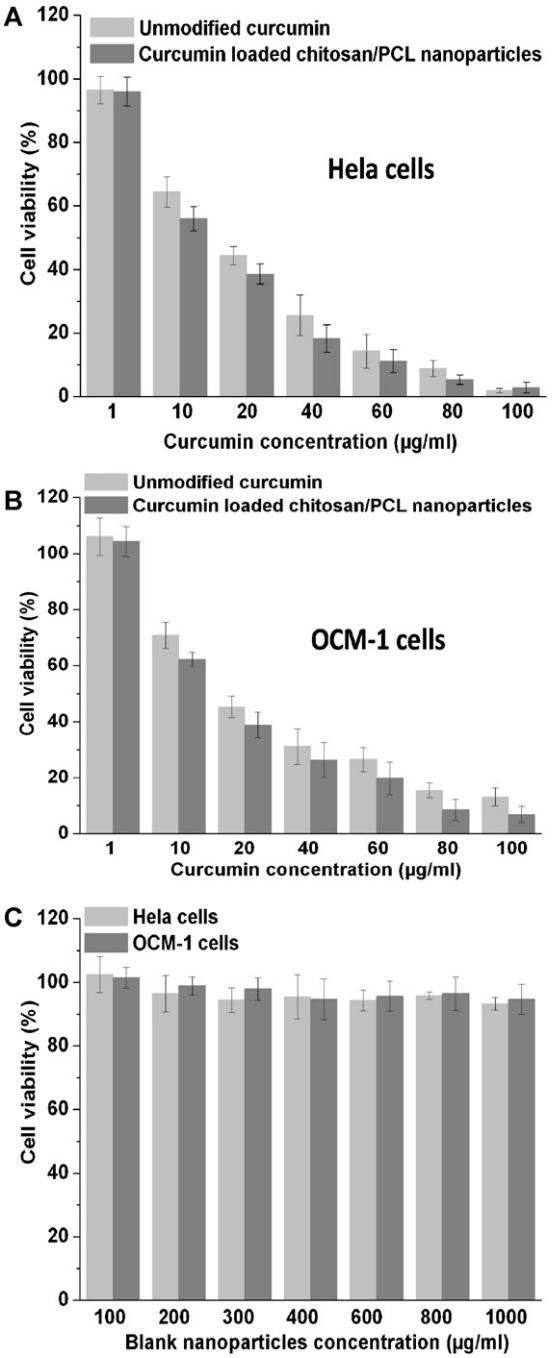

Fig. 5. Dose dependent cytotoxicity of unmodified curcumin, blank chitosan/PCL nanoparticles and curcumin loaded chitosan/PCL nanoparticles against Hela cells and OCM-1 cells after 48 h co-incubation. The data represent the mean ± SD, $n = 6$.

result suggests that curcumin loaded chitosan/PCL nanoparticles exhibit a continuous and slow release behavior for curcumin.

3.5. In vitro cytotoxicity study

To investigate whether the developed nanoparticles could be used for delivering highly hydrophobic drugs such as curcumin to Hela cells and OCM-1 cells, a comparative cytotoxicity study of the effects of unmodified curcumin, curcumin loaded chitosan/PCL nanoparticles and blank chitosan/PCL nanoparticles were performed. The cells were exposed to a range of experimental concentration of either unmodified curcumin or curcumin loaded

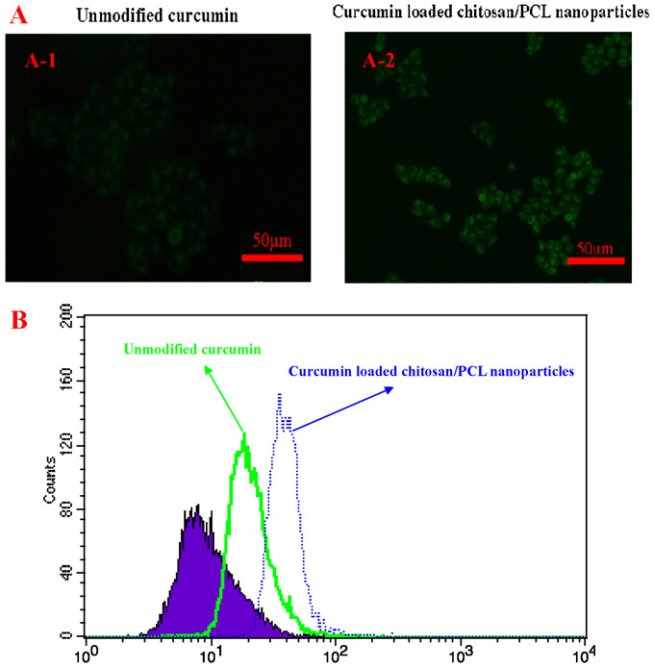

Fig. 6. (A) Fluorescence microscopic images of Hela cells incubated with 10 μg/ml of curcumin at 37 °C for 3 h. (B) Flow cytometry study of Hela cells incubated with 10 μg/ml of curcumin at 37 °C for 3 h.

chitosan/PCL nanoparticles and co-incubated for 48 h. As presented in Fig. 5A and B, the cytotoxicity of curcumin loaded chitosan/PCL nanoparticles was slightly higher than that of unmodified curcumin at the tested concentrations ranging from 1 μg/ml to 100 μg/ml in both Hela cells and OCM-1 cells (but there was no significant difference between two formulations). This similar pattern of cytotoxicity against OCM-1 cells was also observed that the curcumin loading chitosan/PCL nanoparticles exhibited slight higher cytotoxicity compared to unmodified curcumin. Additionally, no obvious cytotoxicity against Hela cells and OCM-1 cells was observed with blank chitosan/PCL nanoparticles at all tested concentrations ranging from 100 μg/ml to 1000 μg/ml (Fig. 5C), suggesting that the blank chitosan/PCL nanoparticles have no cytotoxicity at the normal concentration and show good biocompatibility.

3.6. *In vitro* cell uptake study

Taking the advantage of photochemical properties of curcumin, the intracellular uptake of unmodified curcumin and curcumin loaded chitosan/PCL nanoparticle was observed by fluorescence microscope. As presented in Fig. 6A, it clearly observed that the cells treated with curcumin loaded chitosan/PCL nanoparticle showed profound fluorescence intensity compared with the cells treated with unmodified curcumin at concentration of 10 μg/ml after 3 h incubation. This result indicated that the curcumin loaded chitosan/PCL nanoparticles was more effective in the enhancement of uptake of curcumin. Whereas, the cytotoxicity of curcumin loaded chitosan/PCL nanoparticles did not showed comparable cytotoxic effect with respect to unmodified curcumin at the same dose, which might be induced by that the sustained release of curcumin from nanoparticles. Similar results were also observed by Mohanty and Sahoo (2010) where they demonstrated that the cellular uptake of curcumin could be greatly improved by encapsulated curcumin into MPEG-PCL polymer in PANC-1 cells. Furthermore, the fluorescence intensity was also quantitatively detected by a flow cytometry. It clearly observed that the curcumin loaded chitosan/PCL nanoparticles could effectively improve the cell uptake of curcumin as compared with that of unmodified curcumin. Therefore, the curcumin loaded chitosan/PCL nanoparticles showed comparable cellular uptake of curcumin with respect to unmodified curcumin at the same dose might be a better candidate for cancer therapy.

4. Conclusion

In this study, a novel formulation of curcumin loaded chitosan/PCL nanoparticles was developed and characterized. The obtained curcumin loaded chitosan/poly(ε-caprolactone) (chitosan/PCL) nanoparticle diameter was varied between 220 nm and 360 nm and zeta potential was varied between +30 mV and 0 mV as the function with pH value. Meanwhile, the encapsulated curcumin could be slowly released from nanoparticles as assessed by *in vitro* release study. *In vitro* cellular uptake study revealed that encapsulated of curcumin into chitosan/PCL nanoparticle could greatly enhance the cell uptake of curcumin as compared to unmodified curcumin after 3 h co-incubation. As a result, it was clearly observed that blending PCL nanoparticles with bio-adhesive polymer chitosan resulted in favorable drug loading and release profiles as well as good cellular interaction and *in vitro* anticancer efficacy might be suitable for delivering curcumin.

Acknowledgments

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