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Research Paper

Novel chitosan derivative for temperature and ultrasound dual-sensitive liposomal microbubble gel

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ABSTRACT

In this study, a novel liposome-loaded microbubble gel based on *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan (NCHOSC) was designed. The structure of the NCHOSC was characterized by FTIR and ^{1}H NMR. The liposomal microbubble gel based on NCHOSC with a high encapsulation efficiency of curcumin was formed and improved the solubility of curcumin. The diameter of most liposomal microbubble was about 950 nm. The temperature-sensitive CS/GP gel could be formulated at room temperature and would form a gel at body temperature. Simultaneously, the ultrasound-sensitive induced release of curcumin was 85% applying ultrasound. The results of cytotoxicity assay indicated that encapsulated curcumin in Cur-LM or Cur-LM-G was less toxic. The anti-tumor efficacy in vivo suggested that Cur-LM-G by ultrasound suppressed tumor growth most efficiently. These findings have shed some light on the potential NCHOSC material used to liposome-loaded microbubble gel for temperature and ultrasound dual-sensitive drug delivery.

1. Introduction

Curcumin, a natural diphenolic compound extracted from the rhizome of turmeric (*Curcuma longa* L.), which has a long history of use in Traditional Chinese Medicine, is a good candidate for treating cancerous diseases including prostate, breast and colon cancer because of its low nonspecific toxicity to normal cells (Hatcher, Planalp, Cho, Torti, & Torti, 2008; Shishodia, Chaturvedi, & Aggarwal, 2007). However, it has many good properties and it is not widely used for cancer treatment because of its poor aqueous solubility. Due to its poor water solubility, low bioavailability, low gastrointestinal absorption and rapid metabolism, curcumin is not extensively used for tumor therapy (Pan, Huang, & Lin, 1999).

Microbubble with a few microns in size was generally full of a hydrophobic gas and was fabricated with a surfactant shell such as polymer to improve its shelf life and circulation time in blood. Due to the different density between the gas core of the liposomal microbubble and the fluid around, microbubble began to swing when sent to high frequency (1–10 MHz) ultrasound which become more violent which resulted in ruin of the microbubble (Newman & Bettinger, 2005) The nonlinear acoustic echoes were emerged with the microbubble destruction. This phenomenon was valuable in contrast enhanced ultrasound imaging and drug delivery (Ferrara, Pollard, & Borden, 2007; Mehier-Humbert, Bettinger, Yan, & Guy, 2005). Liposomal microbubble was also reported in gene delivery system which suggested an efficient approach for delivering plasmid DNA or siRNA into cells (Negishi et al., 2008; Suzuki, Takizawa, Negishi, Utoguchi, & Maruyama, 2008).

Our aim was to develop an injectable drug reservoir to provide sustained, temperature and ultrasound dual-sensitive curcumin-loaded liposomal microbubble gel drug delivery. A novel water-soluble chitosan derivatives *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan (NCHOSC) were synthesized with negative charges of sulfonate group as hydrophilic moieties, and cholesterol as hydrophobic moieties. Compared with *N*-octyl-*O*-sulfate chitosan (NOSC; Zhang, Ping, & Zhang, 2004), NCHOSC could be easily modified to liposome because of the cholesterol moieties.

In the previous study (Chen et al., 2012), we developed thermosensitive poloxamer gel for vaginal delivery. However, in this study we developed a biocompatible chitosan/glycerol phosphate (CS/GP) gel as a carrier for liposomal microbubble which was liquid at room temperature but gels at 37 °C under pH 7.4. In this study, we aimed to develop *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan

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(NCHOSC) which were carried to modified liposomal microbubble (LB) to enhance the property and blood-contacting stability. The NCHOSC modified LB was fabricated into the CS/GP thermosensitive gel. At the same time, the thermosensitive gel systems composed of CS/GP form a thermogel at body temperature and to degrade in an ultrasound environment.

Consequently, with this in mind, we prepared temperature and ultrasound dual-sensitive curcumin-loaded liposomal microbubble gel based on NCHOSC. The dual-sensitive curcumin-loaded liposomal microbubble gel was evaluated in terms of physicochemical properties, in vitro release, cytotoxicity and anti-tumor efficacy.

2. Materials and methods

Chitosan was provided by the Shanghai Lanji Co. Ltd., China with deacetylation degrees of 97% and viscosity average molecular weight of 65,000 D. Curcumin (Cur) was supplied by Yantai Science & Biotechnology Co. Ltd. (China). Soybean phosphatidylcholine (S100PC) was purchased from Dongshang Co. Ltd. (Shanghai, China). Cholesterol (Chol) and succinic anhydride were obtained from China Medicine Shanghai Chemical Reagent Corporation (Shanghai, China). Dicyclohexyl carboimide (DCC), 4-(dimethyl amino) pyridine (DMAP), and MTT were obtained from Sigma Chemical Co. (MO, USA). All other reagents were of analytical grade and supplied by Sinopharm Group Chemical Reagent Corp. MCF-7 human breast cancer cell line and hepatoma solidity (Heps) cell were obtained from the Yantai Science & Biotechnology Co. Ltd. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin G and 0.1 mg/mL streptomycin). Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2.

Five-week-old female BALB/c mice were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). All the animals were pathogen-free and allowed to access food and water freely. All the animal experiments were in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

2.1. Synthesis and characterization of NCHOSC

The synthesis of NCHOSC was shown in Scheme 1. Cholesterol (4.0 g) and succinic anhydride (3.0 g) were dissolved in CH_2Cl_2 (60 mL) with DMAP (0.2 g) as the catalyst. The unreacted succinic anhydride was removed by filtration and the CH_2Cl_2 was removed by rotary evaporation. The residue was washed with water and dried overnight. Chitosan (1.0 g) was dissolved in 65 mL methanol with stirring at 30 °C, then cholesteryl hemisuccinate (1.0 g) was added. After 24 h stirring, the reaction solution was neutralized with 2 N hydrochloric acid and the product was precipitated with methanol. The precipitate was filtered and repeatedly washed with methanol and water. The product was dried under vacuum at 60 °C overnight. *N*-cholesteryl hemisuccinate chitosan (0.5 g) suspended in *N*,*N*-dimethylformamide (DMF) (40 mL) was magnetically stirred overnight. Chlorosulfonic acid (20 mL) was added dropwise into DMF (40 mL) with stirring at 0 °C for 24 h. The reaction solution was neutralized with 20% NaOH until pH 7.0, and the filtered solution was dialyzed in water and lyophilized NCHOSC powder was obtained. The chemical structure and substitution degree of the derivative were determined by FT-IR, H NMR.

2.2. Preparation of Cur-loaded liposome microbubble based on NCHOSC

Cur-loaded liposomal microbubble based on NCHOSC (Cur-LM) was prepared by dissolving curcumin in the organic phase of chloroform and methanol (1:3, v/v) and mixing with S100PC, cholesterol, and NCHOSC (90:10:3). The organic phase was removed at 40 °C in a rotary flask. The flask was evaporated under reduced pressure. The dry lipid formed was hydrated with phosphate-buffered saline (pH 7.4). The liposome was filtered through 0.22 μm filters to isolate the unencapsulated curcumin. Liposomal microbubble was prepared from liposomes and perfluoropropane gas. 5 mL vials containing 2 mL of liposome (2 mg/mL) were filled with perfluoropropane gas. The 7.5 mL gas was sparged through the aqueous medium, and performed for 30 s with a bath-type sonicator with high power sonication (power setting dialed to 10/10; 33 W). Then liposomal microbubble was put into a water bath with 10 mL PBS. The Cur-LM was filtered through 0.2 mm polycarbonate filters and stored at 4 °C until use. Curcumin-loaded liposomal microbubble without NCHOSC (control) was also prepared as the control.

2.3. Stability studies

Because NCHOSC in the formulation is to increase the stability of Cur-LM, the particle size plays an important role in the in further studies. However, it is also important to study the stability of the Cur-LM. The stability of the Cur-LM was studied by testing the aggregation of Cur-LM in PBS, or PBS with 50% FBS at 37 °C. The change of the mean particle size was measured before and after 2 h, 12 h, 24 h incubation Cheng, Yang, and Lin (2011). The particle sizes of the Cur-LM were measured with a particle sizer 3000HS (Malvern Instruments, UK) and analyzed by the Zetasizer 3000H (Malvern software).

2.4. Encapsulation efficiency of Cur-LM

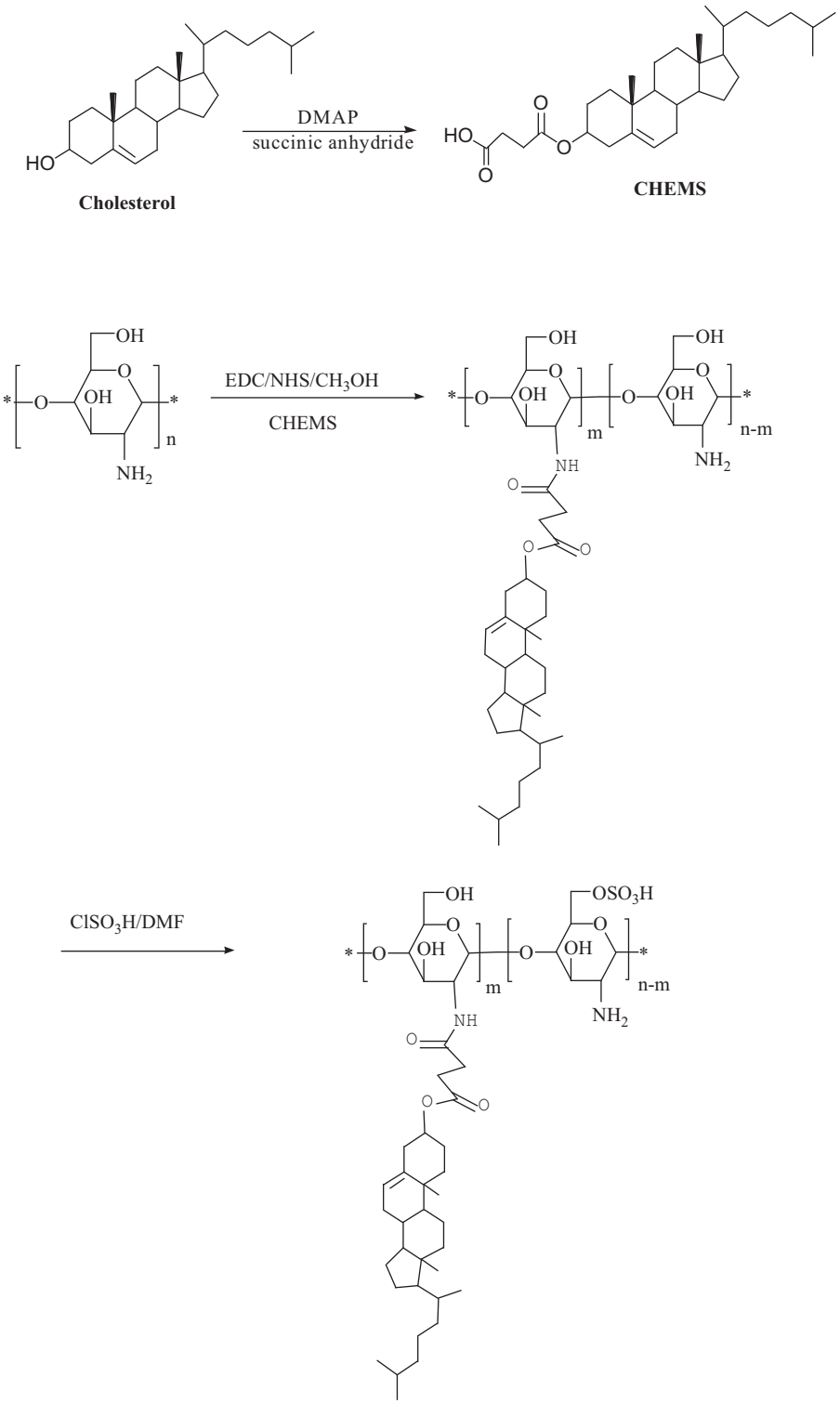
The entrapment efficiency was defined as the ratio of the amount of curcumin encapsulated in the Cur-LM to that of the total curcumin in the Cur-LM dispersion. Cur-LM was treated with a 20-fold volume of methanol to disrupt the structure of the Cur-LM. The amount of encapsulated curcumin was measured using reverse-phase HPLC. The mobile phase was a mixture of acetonitrile and 5% (w/v) of acetic acid at the volume ratio of 51:49 (v/v). The flow rate was set at 1.0 mL/min and the wavelength was at 428 nm. The column was a Kromasil C18 (4.6 mm × 250 mm), the column temperature was 30 °C, and the volume of the sample injected was 20 μL. The entrapment efficiency was determined from three separately prepared liposome suspensions as the mean ± standard deviation (Chen, Jiang, Liu, et al., 2010; Chen, Jiang, Huang, Zhang, & Ping, 2010).

The entrapment efficiency was calculated from the following equation:

$$\mathrm{EE}\,(\%) = \frac{\text{amount of Cur in LM}}{\text{amount of total Cur}} \times 100$$

2.5. Preparation of dual-sensitive gels

Dual-sensitive gels were prepared by the addition of CS and GP at various ratios, from 1:1 to 7:1. Chitosan (0.1 g) was dissolved in 0.1 M acetic acid and the clear 2% (w/v) chitosan solution was obtained. GP (0.56 g/mL) solution was added into the chitosan solution drop by drop under stirring and stored at 4 °C. For dual-sensitive gels, a vial containing Cur-LM-NCHOSC dispersions and CS/GP with a magnetic bar and each formulation was placed in a water bath. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation (Chen et al., 2012). The dual-sensitive gels were filtered through 0.2 mm polycarbonate filters and stored at 4 °C until use.



Scheme 1. Synthesis of *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan (NCHOSC).

2.6. Ultrasound-sensitive induced release in vitro

The in vitro release of Cur was determined from dual-sensitive gels using a dialysis bag placed in a sealed vial with constant shaking. The gel formulation (1 mg of Cur) was packed into the dialysis bags sealed with closures of 50 mm. The dialysis bags containing dual-sensitive gels were placed in a water bath (37 °C) until gel formed. The release medium was 50 mL of 0.1% SDS providing sink conditions for Cur. The medium was maintained at 37 °C and shaken at 100 rpm. At various time intervals, the gels were subjected to ultrasound with 1 MHz ultrasound (5 pulses, 100 ms, 7 MPa peak negative acoustic pressure) (Postema, van Wamel, Lancée, & de Jong, 2004), 1 mL of dissolution fluid was collected. The sample was filtrated and analyzed by a reverse-phase HPLC method. 20 μL of this solution was used to determine the amount of curcumin released after different time intervals (Ferrara et al., 2007; Klibanov, Shevchenko, Raju, Seip, & Chin, 2010).

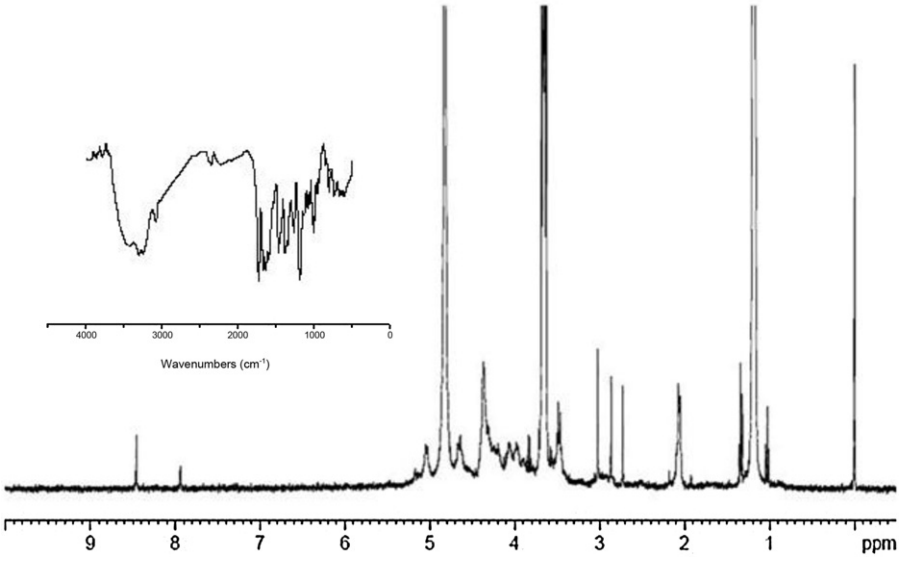


Fig. 1. IR and ^{1}H NMR spectra of NCHOSC.

2.7. Cytotoxicity assay

To evaluate the cytotoxicity of the dual-sensitive curcumin-loaded liposomal microbubble gel by MTT assay, MCF-7 cells were seeded at a density of 1 × 104 cells/well in a 96-well plate overnight. 100 μL of Cur solution (1 mg/mL), the curcumin-loaded liposomal microbubble (Cur-LM), curcumin-loaded liposomal microbubble gel (Cur-LM-G) and free Cur and PBS were added to the each well and incubated overnight. After incubation, 100 μL of 5 mg/mL MTT in DMEM was added to each well and then incubated for 3 h at 37 °C. 100 μL of MTT solution was added with dissolving the formazan crystal. After shaking for 20 min, the absorbance was measured at 570 nm using a micro-plate reader (Chen et al., 2012).

2.8. In vivo assay

The BALB/c mice model was carried out with inoculation of 1×10^7 hepatoma solidity (Heps) cell at the right flank of the mice using a 1.0 mL syringe. When the tumor volume grow about 100–300 mm^3, 0.1 mL dual-sensitive curcumin-loaded liposomal microbubble gel (Cur-LM-G) were injected into the tumor. Then ultrasound was applied to the tumor for a 1 min to ultrasound (6 W/cm^2, 10 s pulse 2 s on/2 s pulse off) every other day. After 2 weeks, all the mice were weighed and sacrificed followed by separation and measurement of the tumor. Normal saline and without ultrasound were injected for the control groups. Each individual tumor size was measured with a caliper and the tumor volume was calculated every other day using the following equation: $(W^2 \times L)/2$, where W is the tumor measurement at the widest point and L is the tumor dimension at the longest point (Chen, Jiang, Liu, et al., 2010).

2.9. Statistical analysis

Statistical comparisons were performed by Student's t-test for two groups, and one-way ANOVA for multiple groups. $P < 0.05$ was considered to be indicative of statistical significance.

3. Results and discussion

3.1. Synthesis and characterization of NCHOSC

The structure change of NCHOSC was confirmed by FT-IR spectra (Fig. 1). The result showed new peaks at 2965, 2842, 1599, 1576, 1503, 910–665 cm^{-1} attributed to cholesteryl hemisuccinate chain and 1260 and 1221 cm^{-1} assigned to the sulfate group. The signal from the —NH_2 group at 1590 cm^{-1} disappeared. These results suggest that sulfate group and cholesteryl hemisuccinate group were part of the NCHOSC structure. Fig. 1. showed the ^{1}H NMR spectra of NCHOSC. The proton signals at 2.58–2.80, 4.62–4.65 and 5.37 ppm are assigned to cholesteryl hemisuccinate group which can be found in the spectrum of NCHOSC. Some new characteristic peaks at 3.75–3.30, 2.68 and 2.78 ppm present due to partial sulfonation in the spectrum of NCHOSC.

3.2. Preparation and characterization of Cur-LM

The curcumin-loaded liposomal microbubble (Cur-LM) became cloudier than the curcumin-loaded liposome (Cur-L). The diameter of most Cur-LM is less than 1 μm, and the average diameter is about 950 nm. In addition, we confirmed by means of microscopy that perfluoropropane gas was entrapped within the liposome microbubble (Fig. 2). The particle size, zeta potentials and entrapment efficiency of Cur-LM would be important factors for the ultrasound triggered drug delivery. As shown in Table 1, the mean particle size of Cur-LM was about 950 nm. The mean zeta potential was −28.7 ± 3.8 mV. The entrapment efficiency was determined to be above 90%.

3.3. Stability studies

The particle size distribution of the Cur-LM in this study showed that there was no significant change when incubated in PBS or PBS with 50% FBS (Table 2), suggesting that NCHOSC in the formulation could increase the stability of Cur-LM. It is interesting to note that the particle sizes of Cur-LM were almost constant for up to 24 h, which could be used for further studies in vitro and in vivo.

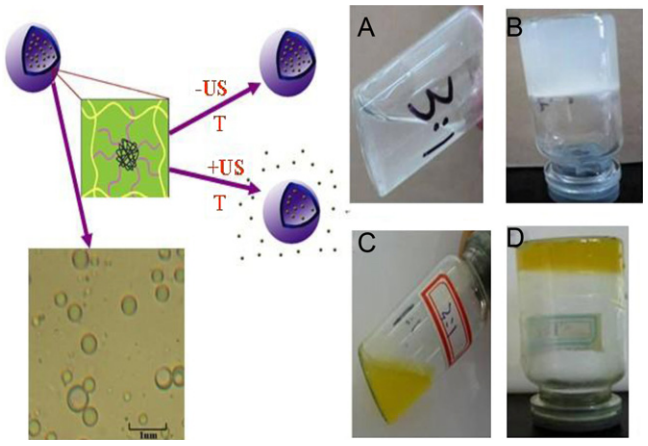


Fig. 2. A schematic diagram of the strategy of Cur-LM-G and exterior morphology studies of blank liposome microbubble gel: under the gelation temperature (A) and gelation (B); Cur-LM-G: under the gelation temperature (C) and gelation (D).

Table 1
The physicochemical parameters of Cur-LM.

Parameters	Control	Cur-LM
Entrapment efficiency (%)	93.8 ± 3.0	92.9 ± 2.6
Particle size (nm)	962.5 ± 6.3	952.6 ± 5.4
Zeta potential (mV)	−2.5 ± 3.9	−28.7 ± 3.8

Table 2
Stability of Cur-LM with change of particle sizes with different media at 37 °C.

	Time (h)	PBS	PBS + FBS
Cur-LM	2	955.8 ± 6.5	958.9 ± 7.3
	12	968.2 ± 5.3	975.6 ± 6.7
	24	972.6 ± 6.2	978.1 ± 7.0

3.4. Preparation and characterization of dual-sensitive gels

In the previous study (Chen et al., 2012), a thermosensitive poloxamer gel for vaginal delivery was characterized with FT-IR and DSC which was similar with dual-sensitive curcumin-loaded liposomal microbubble gel (data were not shown). The blank liposomal microbubble gel was prepared using at various ratios CS/GP. With temperature-sensitive gelling, the concentration of CS/GP (3:1, v/v) was optimal formulation to form gels (Fig. 2). As shown in Fig. 2, the sol–gel of curcumin-loaded liposomal microbubble gels with the optimal formulation of gel/LB (6:1, v/v): under the gelation temperature (C), and gelation (D). The presence of the gel phase around body temperature (37 °C) demonstrated that the CS/GP containing liposomal microbubble could be an interesting and promising candidate for in situ drug delivery system that can be formulated at room temperature and would form a gel at body temperature.

3.5. Ultrasound-sensitive induced release in vitro

The release of curcumin from the dual-sensitive gels was determined by HPLC.

We evaluated whether applying ultrasound on the liposomal microbubble gels results in the release of curcumin from the liposomal microbubble. We measured a significant release increase when curcumin-loaded liposomal microbubble gel was subjected to ultrasound (Fig. 3), which demonstrated that free curcumin was released from the microbubble after ultrasound. The release of curcumin was 85% applying ultrasound on the liposomal microbubble gel, while only 32% without applying ultrasound. The presence of the difference between applying ultrasound or not

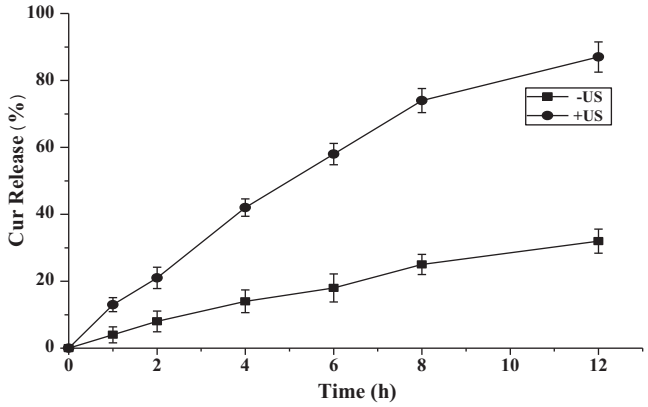


Fig. 3. Percentage of curcumin released from Cur-LM-G with/without the ultrasound (US). Data presented are means ± SD ($n = 3$).

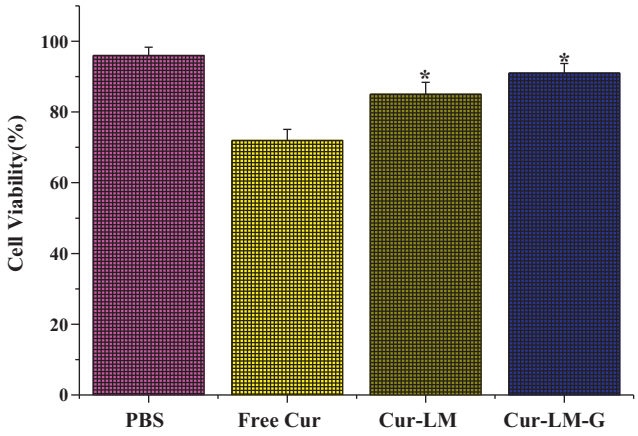


Fig. 4. Cytotoxicity of curcumin formulations. Data represent the mean ± SD ($n = 6$). *$P < 0.05$, compared with the free Cur.

demonstrated that the liposomal microbubble could be an interesting ultrasound-sensitive induced release and a promising candidate for ultrasound-sensitive drug delivery system.

3.6. Cytotoxicity assay

The cytotoxicity of free Cur and formulated Cur was investigated in MCF-7 cells using MTT assays (Fig. 4). As shown in Fig. 5,

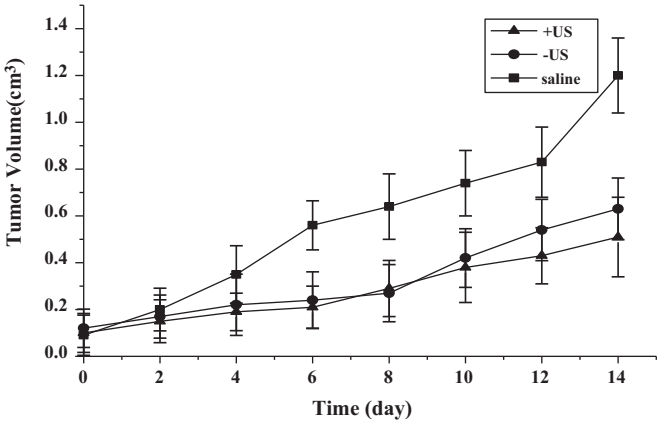


Fig. 5. Effect on the inhibition of tumor growth of Cur-LM-G in BALB/c mice with/without the ultrasound (US). The zero point of *X*-axis indicates the first day of injection. Each data represents the mean ± SD ($n = 6$).

cell viability of free Cur, Cur-LM, Cur-LM-G and PBS alone were 72.8 ± 3.1, 85.2 ± 3.4, 90.3 ± 4.3, and 96 ± 2.3, respectively. The results indicated that encapsulated Cur in Cur-LM or Cur-LM-G was less toxic than the free Cur. It is clear that curcumin encapsulated to Cur-LM and Cur-LM-G could enhance the viability.

3.7. *In vivo assay*

The anti-tumor efficacy of dual-sensitive curcumin-loaded liposomal microbubble gel in vivo was evaluated in tumor-bearing BALB/c mice. As shown in Fig. 5, there was no difference between the groups with or without ultrasound irradiation in 1 week, however, after the next week, there was different. This could be the reason of slow-release of Cur from the liposome-loaded microbubble gel in vivo under the body temperature and ultrasound. The tumor volume of the saline control group was excessively enlarged (>1000 mm^3), while the other groups were much smaller. The ultrasound group suppressed tumor growth most efficiently, followed by the non-ultrasound group ($P < 0.05$). This enhanced anti-tumor activity could be explained by the increased local concentration of Cur near the tumor by ultrasound.

4. Discussion

In this work, we have prepared a novel curcumin-loaded liposomal microbubble gel based on *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan (NCHOSC) which is interesting. In our previous study, the novel water-soluble chitosan derivative NCHOSC was synthesized. Its anticoagulation activity in vitro was determined by an activated partial thromboplastin time (APTT) assay, a thrombin time (TT) assay and a prothrombin time (PT) assay. Results of anticoagulation assays showed NCHOSC significantly prolonged APTT and TT (Fan et al., 2012).

The mechanism of untrasound-induced drug release could be explained that untrasound (US) applied with a very low acoustic pressures could promote liposomal microbubble to move along the axis of the US beam without disrupting the agent (Dayton, Allen, & Ferrara, 2002; Shortencarier et al., 2004). This radiation force can promote the movement and accumulation of liposomal microbubble at a designated area which could be used to increase both specificity of drug delivery to the targeting site and transfection efficiency (Dayton et al., 2002; Shortencarier et al., 2004).

In this study, we aimed to apply NCHOSC to ultrasould-sensitive drug release system for improving its stability and hemocompatibility. Shelma and Sharma prepared a hemocompatible lauroyl sulfated chitosan (LSCS) which could improve the hemocompatibility of chitosan and reduce the protein adsorption on chitosan (Shelma & Sharma, 2011). Compared with this research, we applied NCHOSC to ultrasould-sensitive drug release system for improving its stability and hemocompatibility. Fan et al. reported that the introduction of sulfate groups into the quaternary ammonium chitosan structure could improve its anticoagulant activity obviously (Fan et al., 2012). However, the new application to drug release system, such as liposome, was not reported. Qu et al. and Mo et al. prepared a series of sulfonate chitosan *N*-octyl-*O*-sulfate chitosan (NOSC) in micellar or liposomal delivery (Mo, Xiao, Sun, Zhang, & Ping, 2011; Mo, Jin, et al., 2011; Qu, Yao, Zhang, Wu, & Ping, 2009; Qu, Wu, Yin, & Zhang, 2012). However, there was not reported research about novel chitosan for the hemocompatible activity. There was also no further application research to increase stability of nano-drug delivery system.

In this study, the key feature of gel system is the temperature and ultrasound dual-sensitive system which is the combination of injectability and mechanical integrity (Epstein-Barash et al., 2010). Each component served a specific aim: the NCHOSC modified liposome carried the model drugs and promoted the stability, prevented their premature release, the microbubble enhanced the drug release, and the temperature-sensitive gel maintained both particles in body temperature could be affected by US (Epstein-Barash et al., 2010).

5. Conclusions

In this work, we have prepared a novel curcumin-loaded liposome microbubble gel based on *N*-cholesteryl hemisuccinate-*O*-sulfate chitosan (NCHOSC). The liposomal microbubble based on NCHOSC with a high encapsulation efficiency of curcumin was formed and improved the solubility of curcumin. The chemical structure of NCHOSC was characterized by FTIR, ^1H NMR. As ultrasound guided drug delivery carrier, liposome-loaded microbubble and gels were characterized, containing in vitro studies, cell assay and in vivo assay using curcumin as a model drug. The diameter of most liposomal microbubble is about 950 nm. The temperature-sensitive CS/GP gel containing liposomal microbubble can be formulated at room temperature and would form a gel at body temperature. Simultaneously, the ultrasound-sensitive induced release of curcumin was 85% applying ultrasound. The results of cytotoxicity assay indicated that encapsulated curcumin in Cur-LM or Cur-LM-G was less toxic than the free curcumin. The anti-tumor efficacy in vivo suggested that Cur-LM-G by ultrasound suppressed tumor growth most efficiently. These results indicated that dual-sensitive curcumin-loaded liposomal microbubble gel could be a potential temperature and ultrasound dual-sensitive drug carrier in local drug delivery system.

Conflicts of interest

The authors report no conflicts of interest in this work.

Acknowledgments

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References

Chen, D., Jiang, X., Huang, Y., Zhang, C., & Ping, Q. (2010). pH-sensitive mPEG-Hz-cholesterol conjugates as a liposome delivery system. *Journal of Bioactive and Compatible Polymers*, *25*, 527–542.

Chen, D., Jiang, X., Liu, J., Jin, X., Zhang, C., & Ping, Q. (2010). In vivo evaluation of novel pH-sensitive mPEG-Hz-chol conjugate in liposomes: Pharmacokinetics, tissue distribution, efficacy assessment. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology*, *38*, 136–142.

Chen, D., Sun, K., Mu, H., Tang, M., Liang, R., Wang, A., et al. (2012). pH and temperature dual-sensitive liposome gel based on novel cleavable mPEG-Hz-CHEMS polymeric vaginal delivery system. *International Journal of Nanomedicine*, *7*, 2621–2630.

Cheng, Y. H., Yang, S. H., & Lin, F. H. (2011). Thermosensitive chitosan–gelatin–glycerol phosphate hydrogel as a controlled release system of ferulic acid for nucleus pulposus regeneration. *Biomaterials*, *32*, 6953–6961.

Dayton, P. A., Allen, J. S., & Ferrara, K. W. (2002). The magnitude of radiation force on ultrasound contrast agents. *Journal of the Acoustical Society of America*, *112*, 2183–2192.

Epstein-Barash, H., Orbey, G., Polat, B. E., Ewoldt, R. H., Feshitan, J., Langer, R., et al. (2010). A microcomposite hydrogel for repeated on-demand ultrasound-triggered drug delivery. *Biomaterials*, *31*, 5208–5217.

Fan, L., Wu, P., Zhang, J., Gao, S., Wang, L., Li, M., et al. (2012). Synthesis and anticoagulant activity of the quaternary ammonium chitosan sulfates. *International Journal of Biological Macromolecules*, *50*, 31–37.

Ferrara, K., Pollard, R., & Borden, M. (2007). Ultrasound microbubble contrast agents: Fundamentals and application to gene and drug delivery. *Annual Review of Biomedical Engineering*, *9*, 415–447.

Hatcher, H., Planalp, R., Cho, J., Torti, F. M., & Torti, S. V. (2008). Curcumin: From ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences*, *65*, 1631–1652.

Klibanov, A. L., Shevchenko, T. I., Raju, B. I., Seip, R., & Chin, C. T. (2010). Ultrasound-triggered release of materials entrapped in microbubble-liposome constructs: A tool for targeted drug delivery. *Journal of Controlled Release*, *148*, 13–17.

Mehier-Humbert, S., Bettinger, T., Yan, F., & Guy, R. H. (2005). Plasma membrane poration induced by ultrasound exposure: Implication for drug delivery. *Journal of Controlled Release*, *104*, 213–222.

Mo, R., Jin, X., Li, N., Ju, C., Sun, M., Zhang, C., et al. (2011). The mechanism of enhancement on oral absorption of paclitaxel by *N*-octyl-*O*-sulfate chitosan micelles. *Biomaterials*, *32*, 4609–4620.

Mo, R., Xiao, Y., Sun, M., Zhang, C., & Ping, Q. (2011). Enhancing effect of *N*-octyl-*O*-sulfate chitosan on etoposide absorption. *International Journal of Pharmaceutics*, *409*, 38–45.

Negishi, Y., Endo, Y., Fukuyama, T., Suzuki, R., Takizawa, T., Omata, D., et al. (2008). Delivery of siRNA into the cytoplasm by liposomal bubbles and ultrasound. *Journal of Controlled Release*, *132*, 124–130.

Newman, C. M., & Bettinger, T. (2005). Gene therapy progress and prospects: Ultrasound for gene transfer. *Gene Therapy*, *14*, 465–475.

Pan, M. H., Huang, T. M., & Lin, J. K. (1999). Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metabolism and Disposition*, *27*, 486–494.

Postema, M., van Wamel, A., Lancée, C. T., & de Jong, N. (2004). Ultrasound-induced encapsulated microbubble phenomena. *Ultrasound in Medicine and Biology*, *30*, 827–840.

Qu, G., Wu, X., Yin, L., & Zhang, C. (2012). *N*-octyl-*O*-sulfate chitosan-modified liposomes for delivery of docetaxel: Preparation, characterization, and pharmacokinetics. *Biomedicine & Pharmacotherapy*, *66*, 46–51.

Qu, G., Yao, Z., Zhang, C., Wu, X., & Ping, Q. (2009). PEG conjugated *N*-octyl-*O*-sulfate chitosan micelles for delivery of paclitaxel: In vitro characterization and in vivo evaluation. *European Journal of Pharmaceutical Sciences*, *37*, 98–105.

Shelma, R., & Sharma, C. P. (2011). Development of lauroyl sulfated chitosan for enhancing hemocompatibility of chitosan. *Colloids and Surfaces B: Biointerfaces*, *84*, 561–570.

Shishodia, S., Chaturvedi, M. M., & Aggarwal, B. B. (2007). Role of curcumin in cancer therapy. *Current Problems in Cancer*, *31*, 243–305.

Shortencarier, M. J., Dayton, P. A., Bloch, S. H., Schumann, P. A., Matsunaga, T. O., & Ferrara, K. W. (2004). A method for radiation-force localized drug delivery using gas-filled lipospheres. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, *51*, 822–831.

Suzuki, R., Takizawa, T., Negishi, Y., Utoguchi, N., & Maruyama, K. (2008). Effective gene delivery with novel liposomal bubbles and ultrasonic destruction technology. *International Journal of Pharmaceutics*, *354*, 49–55.

Zhang, C., Ping, Q., & Zhang, H. (2004). Self-assembly and characterization of paclitaxel-loaded *N*-octyl-*O*-sulfate chitosan micellar system. *Colloids and Surfaces B: Biointerfaces*, *25*, 69–75.