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Rectorite-intercalated nanoparticles for improving controlled release of doxorubicin hydrochloride

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ABSTRACT

Controlled release of drugs has been widely researched in biomedical area. Nanoparticles (NPs) as ideal drug carriers are often used to facilitate improvements in the therapeutic index of drugs. In this study, natural polymers carboxymethyl chitosan (CMC) and lysozyme (LY) were mixed to prepare CMC-LY NPs by electrostatic self-assembly interactions. In addition, layered silicate rectorite (REC) was introduced into NPs to explore the effect on the encapsulation efficacy and controlled release of doxorubicin hydrochloride (DOX). It was confirmed that the average size of NPs increased with the addition of REC, and the interlayer distance of REC in NPs was enlarged because of the intercalation with polymer chains. Besides, the encapsulation efficiency and loading capacity of DOX in NPs increased markedly with the accretion of REC. The incorporation of REC into NPs could reduce the initial burst release and prolong the therapeutic time. Such results suggest that the REC-intercalated NPs are promising anticancer drug carriers for efficient cancer therapy.

1. Introduction

Doxorubicin hydrochloride (DOX), one of the most effective agents for tumor therapy, has been widely used in clinical applications of anticancer therapy [1]. However, it can be quickly dissolved in tissue fluid owing to its high solubility, which could lead to initial burst release [2]. In addition, high concentrations of drugs can lead to toxic side effects, which are harmful to patients [3]. Therefore, it is urgent for seeking appropriate carriers to improve the effectiveness of drug therapies and reduce the side effects.

Many carriers such as nanoparticles (NPs), nanofibers, liposomes, hydrogels and nanomicelles, have been developed and applied in drug delivery systems. NPs, a kind of important carrier for drugs, plays a vital role in the field of drug delivery [4]. Among all kinds of NPs, natural polymer-based NPs have several unique advantages such as good biological compatibility and nontoxicity, which are preferable for drug carriers. Natural proteins and polysaccharides are both charged molecules, they could attract each other by electrostatic self-assembly interactions, further agglomerated into NPs [5,6]. Chitosan is one of the most abundant polysaccharides which is synthesized by the deacetylation of chitin [7,8]. Chitosan as a nature polymer has been widely studied in biomedical applications such as tissue engineering, wound healing and drug delivery [9–12]. However, chitosan can be just dissolved in acid solution, which greatly restricts its application [13]. Therefore, various chitosan derivatives have been developed to increase its water solubility. Carboxymethyl chitosan (CMC), a water soluble derivative of chitosan, is not only soluble in water, but also possesses excellent properties such as low toxicity and good biocompatibility [7]. It has been widely used in biomedical fields [14–16]. Lysozyme (LY) is a kind of globular protein with a molecular weight of 14.3 kDa and the isoelectric point value of 10.7 [17]. There are many investigations in using LY as a model medicine to establish biodegradable drug delivery system [18,19]. Besides, LY can also be applied for fabricating NPs due to its good gelation ability, static electricity and outstanding emulsification [20,21].

Organic–inorganic composites have been reported as a nontoxic and biodegradable scaffold for tissue engineering and drug delivery [22]. Wang et al. had fabricated Chitosan/organic rectorite nanocomposite films and studied the drug delivery behavior of

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composite films [23]. Xu et al. prepared organic-inorganic hybrid nanocomposites which could be a promising ocular drug delivery system owing to the enhanced ocular bioavailability and better corneal permeability [24]. Besides, in our previous research, Tu et al. have prepared quaternized chitosan/bovine serum albumin/rectorite NPs for the controlled release of drugs [25]. It can be found that the activity of drug controlled release of polymers could be enhanced by mixing rectorite (REC) into carriers [25,26] because of the high surface area, chemical and mechanical stability, layered structure and easily formulated intercalation of REC [27]. In addition, it has been proved that REC can be applied in food packing and drug delivery owing to its low cytotoxicity [28,29].

In the present study, CMC and LY were combined to fabricate NPs spontaneously by electrostatic self-assembly interactions. NPs were fabricated with one-step synthesis by a simple and green method. The diameter distribution, morphology, composition, encapsulation efficiency (EE) and loading capacity (LC) of NPs were investigated. The *in vitro* release properties of DOX in NPs had also been evaluated. Besides, REC was introduced in NPs to investigate the impact on the capability of facilitating sustained release of DOX.

2. Materials and methods

2.1. Materials

Chitosan (CS, $M_w = 2.0 \times 10^5$ Da) was obtained from Zhejiang Yuhuan Ocean Biochemical Co., China. Calcium rectorite (Ca^{2+}-REC) was provided by Hubei Mingliu Inc., Co., China. Lysozyme (LY) was purchased from Solarbio Inc., Co., China. DOX ($M_w = 580$ Da) was received from Yuancheng technology Co., China. All other chemicals were of analytical grade unless stated otherwise.

2.2. Preparation of CMC and CMC/REC composites

Carboxymethyl chitosan (CMC) was prepared corresponding to our previous report [30]. 5 g of chitosan was alkalified by 50% NaOH solution (w/w) and then frozen for 24 h at −20 °C. Subsequently, frozen alkali chitosan, 100 mL of isopropyl alcohol and 6 g of chloroacetic acid were added in the three-necked flask. After reacted for 5 h at 60 °C, the mixture was filtered and the residue was dissolved in 250 mL purified water. The pH value of obtained solution was adjusted to 7. Finally, the solution was dialyzed in deionized water at 25 °C for 5 days and then lyophilized to obtain CMC. CMC/REC intercalated composites solutions were prepared according the previous studies [25,31]. The nanocomposites were prepared with CMC/REC weight ratios of 12:1, 6:1 and 3:1, respectively.

2.3. Formation of CMC-LY or CMC/REC-LY NPs

The whole experimentation was carried out in a green and efficient method. The materials for fabricating NPs were natural polymers, even chitosan was extracted from shrimp shell as waste recycling. Besides, there were not any toxic reagents in the preparation process of NPs. In addition, the experimental process was simple with only one step, just by adding LY solution dropwise into CMC solution or CMC/REC composites (Scheme 1). NPs solutions with different mass ratio of CMC and LY were prepared to find the optimum proportion. After determined the optimum weight ratio of CMC to LY, selected the same proportion to prepared CMC/REC-LY NPs by using CMC/REC instead of CMC. Here, the optimum CMC-LY NPs were labeled with NP0. CMC/REC-LY NPs designated as NP121, NP61 and NP31, represented the weight ratios of CMC: REC with 12:1, 6:1 and 3:1, respectively. The DOX-loaded NPs were fabricated *via* adding LY/DOX solutions into CMC/REC composite suspensions.

2.4. Characterization

The particle size, polydispersity (PDI) and ζ-potential of the NPs in aqueous solutions were determined using Malvern Nano 3690 (Malvern Instruments, UK). Each sample was measured three times, the average value and standard deviation were calculated. Transmission electron microscope (TEM, JEM-2100, JEOL, Japan) was used to observe the morphology of NPs. Selected area electron diffraction (SAED, JEM-2100, JEOL, Japan) was applied to investigate the intercalated structure of REC in NPs. Small angle X-ray diffraction (SAXRD) was performed on type D/max-Ra diffractometer (Rigaku Co., Japan) with Cu target and Kα radiation (λ = 0.154 nm). The analysis of the elementary composition of samples were conducted by energy-dispersive X-ray (EDS, Gatan, GENESIS XM2, American), fourier transform infrared (FT-IR, Thermo Nicolet, 170-SX, USA) and X-ray photoelectron spectroscopy (XPS, Kratos, UK).

2.5. Determination of encapsulation efficiency (EE) and loading capacity (LC) of DOX into NPs

The NPs solutions with different composition were centrifuged at 10,000 rpm for 15 min. The concentration of DOX was examined with UV–1780 spectrophotometer (Shimadzu, Japan) at 480 nm [32]. All experiments were conducted in triplicate. The EE and LC were calculated as follows:

$$\mathrm{EE}\,(\%) = \frac{\text{Total amount of added DOX} - \text{free amount of DOX}}{\text{Total amount of added DOX}} \times 100\% \quad (1)$$

$$\mathrm{LC}\,(\%) = \frac{\text{Total amount of added DOX} - \text{free amount of DOX}}{\text{Total amount of lyophilized nanoparticles}} \times 100\% \quad (2)$$

2.6. Measurement of cumulative release of DOX

The sediments obtained from centrifugation were freeze-dried, dispersed into 15 mL of HCl (pH 1.2) and phosphate buffer solution (PBS, pH 7.4), and then stirred in an incubator shaker at 100 rpm under 37 °C. Here, 1 mL of supernatant was extracted for determining the concentration of the released DOX, meanwhile 1 mL fresh HCl or PBS solution was replenished. The amount of DOX remained in dipping solution was examined *via* UV–1780 spectrophotometer at 480 nm. The determine time points were 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h and 48 h. All the tests were carried out in triplicate.

3. Results and discussion

3.1. The DLS results of NPs

Particle size, PDI and scattering light intensity are used to evaluate the results of NPs formed with different weight ratios of CMC: LY. In Fig. 1a, it could be seen that when the ratios changed from 2:1 to 2:3, the average size of the NPs decreased, but increased when the ratios varied from 2:3 to 2:5. The NPs prepared with the ratio of 2:3 exhibited the smallest average diameter (d = 90 ± 13 nm), with the PDI of 0.149 and the count rate of 371, which indicated that the NPs with the weight ratio of 2:3 not only had smaller size and homogeneous distribution but also were strongly steady. The result was identical with the previous report [33]. Therefore, NPs formed