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In situ mineralization of anticancer drug into calcium carbonate monodisperse nanospheres and their pH-responsive release property

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ABSTRACT

In this paper, we facilitated the preparation of uniform calcium carbonate nanospheres and the encapsulation of anticancer drug (Doxorubicin, Dox) in one step by a facile bio-inspired mineralization method at room temperature. Hesperidin (Hesp), a natural originated flavanone glycoside, was introduced as crystallization modifier. The obtained Dox encapsulated $CaCO_3$ nanospheres (Dox@$CaCO_3$-Hesp NSs) having a narrow size range of ~200 nm. The drug loading/release studies reveal that these Dox@$CaCO_3$-Hesp NSs have a drug loading efficiency (DLE) of 83% and drug loading content (DLC) of 14 wt%. Besides, the release of Dox from Dox@$CaCO_3$-Hesp NSs was pH depended. At pH = 7.4, only a small amount (~28%) of Dox was released. While at pH = 5.0, all amount of incorporated Dox was released. Confocal laser scanning microscopy (CLSM) image reveals the Dox@$CaCO_3$-Hesp NSs can internalize the cells. These results suggest the Dox@$CaCO_3$-Hesp NSs can be potentially used to utilize pH-responsive delivery of anticancer drugs.

1. Introduction

Nanomaterials have received much attention in biomedical field, especially in drug detection sensor and drug encapsulation [1–4]. The strategy of preparing nanocarriers while encapsulating guest drug *in situ* is always attractive because it permits loading large quantities of guests [5,6]. Structural design of stimuli-responsive drug delivery system (DDS), such as pH-responsive DDS, is still a challenge [7]. Herein, in this work, we aimed to develop a DDS based on $CaCO_3$ nanospheres for effective loading and pH responsive release of anticancer drug molecules.

Among various reported organic and inorganic carriers, $CaCO_3$ has attracted much attention because of its good biocompatibility and biodegradation [8–13]. $CaCO_3$ based nanospheres will degrade gradually under an acid stimulus, such as tumor tissues and cancer cells, along with anticancer drug releasing [14–21]. By the intrinsic advantage of pH-responsive, $CaCO_3$ nanospheres (NSs) have been known as a smart DDS for anticancer drugs. Till now, popular protocol for $CaCO_3$ based DDS is preparing the carriers first, then loading the drugs by them. Ma and her colleagues' work is a classical example of such two steps strategy [22]. However, DDS got from the two steps protocol is faced two potential defects. One is some guests may only absorb loosely on the outer surface of the carriers, which may cause a loss of drug during circulation in plasma and decrease the treatment efficacy. The other one is that the loading amount is not easy to control.

Therefore, to overcome the two steps' drawbacks, in this paper, we facilitated the preparation of uniform $CaCO_3$ NSs and the encapsulation of Dox in one step by a facile bio-inspired mineralization method at room temperature. As illustrated in Fig. 1a, our protocol is first to make a homogeneous mixed solution of $CaCl_2$, Hesp and Dox. We inferred that those Ca^{2+} can complex with both Dox and Hesp in the solution due to the interactions between Ca^{2+} and the functional groups, *i.e.* —OH or —COOH from Hesp and Dox. Those Ca-Hesp or Ca-Dox complexes may play a key role in the mineralization of products according to the similar reported situations [22–25]. Subsequently, the crystallization of $CaCO_3$ was initiated by gradual diffusion of CO_2 and NH_3 into the mixed solution. Finally, uniform Dox@$CaCO_3$-Hesp NSs were collected by centrifugation from the solution. The drug loading and releasing properties of those Dox@$CaCO_3$-Hesp NSs were also investigated.

2. Experimental

2.1. Materials

Doxorubicin and Hesperidin were purchased from Sigma Chemical Co. (St Louis, MO, USA). $CaCl_2$ (analytical reagent) and $(NH_4)_2CO_3$ (analytical reagent) were purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). Triply distilled deionized water was used during all the applications.

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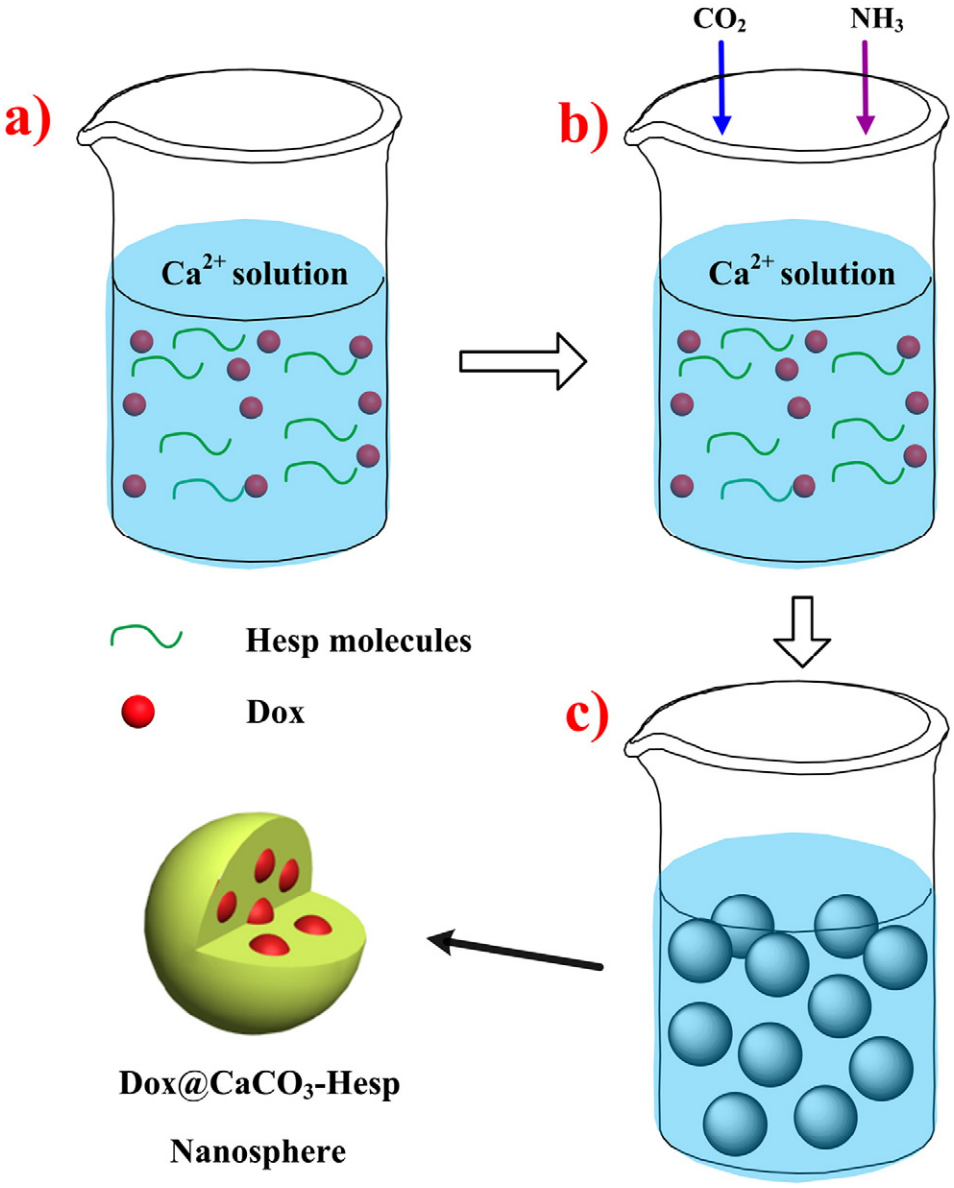

Fig. 1. Schematic preparation process of Dox@$CaCO_3$-Hesp nanospheres.

2.2. Preparation of Dox@$CaCO_3$-Hesp nanospheres

Hesp (60 mg) was dissolved in 20 mL alkaline aqueous solution (pH = 11.0, adjusted by ammonia based on deionized water). Dox (20 mg) was dissolved in 20 mL water. After that, the mineralization was carried out according to the reported similar procedure [26]. A solution of $CaCl_2$ (20 mL, 1 mol/L) was added into a beaker (100 mL), where the as prepared Hesp solution and Dox solution were further added. The mixture was stirred using vortex mixing (IKA, Vortex, Genius 3) to obtain homogeneous solution. Then, the beaker was covered with aluminum foil punctured for several small holes with a needle. A glass petri-dish was filled with crushed ammonium carbonate and was also covered with a piece of punctured aluminum foil. Subsequently, the beaker and the petri-dish were both placed in a closed desiccator at room temperature for 36 h. The vessel was left till prior to harvesting the products. Finally, the collected products were rinsed with deionized water several times and air-dried for further analyses.

In order to investigate the effects of Hesp feed ratio on the formation of $CaCO_3$ nanoparticles, besides 60 mg Hesp, we also set the initial add amount of Hesp at 0, 20, and 200 mg. The following precipitation of $CaCO_3$ nanoparticles adopted the above procedures.

2.3. Characterization

The morphologies of the nanospheres were investigated by scanning electron microscopy (SEM, Hitachi, S4800, Tokyo, Japan) and transmission electron microscopy (TEM, JEM-1200EX, Tokyo, Japan). The determination of Ca/C/O ratios of the sample was performed by a SEM associated energy dispersive X-ray spectroscopy (EDX, Oxford INCA Penta FET ×3) at least twice. To investigate the surface charge of the products, zeta potentials (ζ) were performed on the samples by using a Malvern Nano ZS ZEN3600 instrument. Measurements were averaged over 12 runs using deionized water at pH = 7.0.

The composition of nanospheres was identified by using Fourier transform infrared spectroscopy (FTIR, SHIMADZU, Kyoto, Japan) in the range of 4000 to 400 cm^{-1} with the KBr disk method.

The thermogravimetric data (TG) of the samples were assessed with a NETZSCH STA 449C DSC/DTA-TG analyzer scanning from 30 to 800 °C under an air atmosphere. The samples were weighted and introduced into aluminum pans. The pans were heated at a constant rate of 10 °C/min in nitrogen atmosphere with an empty aluminum pan as the reference probe. The sample mass was in the range of 8–10 mg. All samples were run in duplicate.