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Effect of molecular weight of hyaluronan on zein-based nanoparticles: Fabrication, structural characterization and delivery of curcumin

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ABSTRACT

Zein and hyaluronan with different molecular weights (hyaluronic acid, HA and sodium hyaluronate, SHA: 100, 1000, and 2000 kDa) were used to fabricate the zein-hyaluronan (ZH) nanoparticles by the antisolvent coprecipitation method. With increasing molecular weight of hyaluronan, the particle size, zeta-potential, and turbidity were gradually increased. Zeta-potential and Fourier transform infrared spectroscopy (FTIR) results indicated that electrostatic attraction was the dominant driving force, followed by hydrogen bonding and hydrophobic effects. Circular dichroism and fluorescence spectroscopy results revealed that the secondary structure was changed in zein after its combination with hyaluronan. The uniform spherical zein-HA (100 kDa) nanoparticles (size, 186.4 nm) was designed as a new delivery vehicle for curcumin with the high encapsulation efficiency (95.03%) and loading capacity (3.66%), and curcumin exhibited a better stability of anti- light degradation, and control release in simulate gastrointestinal digestion. This work would have a contribution to the development of novel delivery systems.

1. Introduction

Zein is an alcoholic soluble protein, which is used for fabricating nanoparticles to deliver bioactive compounds (Joye & McClements, 2016). However, zein nanoparticles have poor stability when suffered from acid, base, saline ions, and heat treatment. In order to improve their stability, some biopolymers were used to coat the surface of zein nanoparticles (Chang, Wang, Hu, & Luo, 2017; Joye & McClements, 2016). Moreover, the zein-biopolymer nanoparticles presented a good protective capacity and high encapsulation efficiency (EE) for bioactive compounds (Liang et al., 2015; Luo, Teng, & Wang, 2012). Such as eugenol (Lei & Yue, 2017), resveratrol (Davidov-Pardo, Joye, & McClements, 2015), and curcumin (Dai et al., 2018). Currently, the investigation into development of new delivery systems based on zein and polysaccharides has been becoming a research hotspot.

Previous studies mainly focused on the effects of different polysaccharides on zein-based composite nanoparticles (Chang et al., 2017), such as zein-pectin composite nanoparticles (Hu et al., 2015), zein-chitosan composite nanoparticles (Liang et al., 2015), and zein-propylene glycol alginate composite nanoparticles (Sun, Dai, & Gao, 2017). Another part of the research work investigating into the concentration of polysaccharides on the zein-based composite nanoparticles (Cheng & Jones, 2017; Sun et al., 2017). However, little information about the effects of different molecular weight (M (w)) of a polysaccharide on zein-based composite nanoparticles was available up to now. In our previous study, the M(w) of chitosan had greatly influence on the stability and rheological properties of β-carotene emulsions (Hou et al., 2012). Analogously, a hypothesis was proposed that the M (w) of polysaccharides could affect the structural properties and stability of zein-polysaccharide composite nanoparticles.

Hyaluronan is a functional linear polysaccharide, which occurs in the form of acid (hyaluronic acid, HA) and its salt (sodium hyaluronate, SHA) (Dicker et al., 2014). The molecular structure of hyaluronan is repeat of *D*-glucuronic acid and *N*-acetyl-d-glucosamine (Borzacchiello, Russo, Malle, Schwach-Abdellaoui, & Ambrosio, 2015), so it has different M (w) depending on the number of basic units. Different M (w) of hyaluronan have various biological and physicochemical functions, the low M (w) of hyaluronan (20–200 kDa) are anti-inflammatory, immuno-stimulatory and pro-angiogenic (Dicker et al., 2014), while the high M (w) of hyaluronan (~2000 kDa) have anti-aging, space-filling, and embryonic development (Borzacchiello et al., 2015; Dicker et al., 2014). In addition, hyaluronan is used to fabricate some drug or gene

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delivery systems, such as hyaluronan-chitosan nanoparticles (Almalik et al., 2017; Nazeri et al., 2013), hyaluronan-silk hydrogels (Raia et al., 2017), hyaluronan-curcumin nanogels (Jiang et al., 2016). However, the zein-hyaluronan (ZH) composite nanoparticles made of zein and hyaluronan is scarcely reported until now.

The present work was to explore the effect of hyaluronic M (w) on physicochemical properties, interaction mechanism, and structural feature of ZH nanoparticles. The ZH nanoparticles was designed as a new delivery vehicle of curcumin, to enhance its stability of anti-degradation, and control release in the gastrointestinal digestion. These results from the work would contribute to the development of delivery vehicles for bioactive compounds.

2. Materials and methods

2.1. Materials

Low M (w) (100 kDa), medium M (w) (1000 kDa), and high M (w) (2000 kDa) of hyaluronic acid (HA) and sodium hyaluronate (SHA) (purity, 99%) were obtained from Xi'an Baichuan Company. (Xi'an, China). Zein (95%) was obtained from Gaoyou Company. (Jiangsu, China). Curcumin (Cur, purity, 98%) was obtained from China National Medicine Group (Shanghai, China). Ethanol (99.9%) was bought from Beijing Chemical Company (Beijing, China).

2.2. Fabrication of ZH nanoparticles

ZH nanoparticles were fabricated by the antisolvent coprecipitation (ASCP) method. Briefly, 1.0 g zein was added into 100 mL hyaluronan (2 mg/mL) aqueous ethanol solution (70%, v/v). The solutions were stirred until completely dissolved and then ultrasonic treatment for 20 min. They were injected (20 mL/min) into the 300 mL distilled water with stirring (600 rpm, 20 min). The rotary evaporation was performed (40 °C, −0.1 MPa) to remove ethanol. Individual zein and hyaluronan dispersions were prepared as controls. Part of the samples were stored in refrigerator (4 °C), and the other part of the samples were freeze-dried (−50 °C, 48 h) for solid-state sample characterization.

The low M (w) hyaluronic acid (LHA) and sodium hyaluronate (LSHA); medium M (w) hyaluronic acid (MHA) and sodium hyaluronate (MSHA); high M (w) hyaluronic acid (HHA) and sodium hyaluronate (HSHA) were individually combined with zein and formed the composite nanoparticles. They were termed as ZLHA, ZLSHA, ZMHA, ZMSHA, ZHHA, and ZHSHA, respectively.

2.3. Characterization of typed ZH nanoparticles

2.3.1. Particle size, zeta-potential, turbidity, FTIR and FE-SEM

Particle size, zeta-potential and turbidity measurement of zein, hyaluronan, and ZH nanoparticles were determined with reference to our previous method (Sun et al., 2017). All experiments were performed in triplicate and data were calculated as mean value.

The infrared spectra of samples were measured according to the method of Luo et al. (2012). Measurement parameters: spectral range, 400 − 4000 cm^{-1}; scan times, 11; resolution, 4 cm^{-1}. The micromorphology of ZH nanoparticles were captured by a field emission scanning electron microscopy (FE-SEM, SU8010, Hitachi). Before scanning, a layer of gold was sprayed on the surfaces of samples. FE-SEM measurement: accelerating voltage was 5.0 kV, and magnification factor was 50.0 k.

2.3.2. Fluorescence spectroscopy (FS) and circular dichroism (CD) measurements

FS measurement was carried out according the method of Liu, Ma, Mcclements, & Gao (2017). Measurement parameters: sample concentration, 0.25 mg/mL; spectral range, 290–450 nm; scan speed, 100 nm/min; excitation wavelength, 280 nm; excitation slit width and emission slit width were 10 nm. CD measurement: scanning wavelength was in 190–260 nm, path length was 0.1 cm, recorded speed was set at 100 nm/min, resolution was 0.2 nm, and bandwidth was 2.0 nm. CD data were analyzed using Dichroweb and Selcon3 (Whitmore & Wallace, 2004).

2.3.3. Physical stability

Physical stability was measured refer to the method described by Liu, Wang, Sun, & Gao (2016). LUMiSizer (LUM, Germany) measurement parameters: sample volume was 1.8 mL, temperature was 25 °C, rotational speed was 4000 rpm, time interval was 30 s.

2.4. Delivery characteristics of curcumin (cur)

2.4.1. Fabrication of zein-Cur-hyaluronan composite nanoparticles

1.0 g zein and 50 mg Cur were mixed into 100 mL hyaluronan (2 mg/mL) aqueous ethanol solution (70%, v/v). The solutions were stirred until completely dissolved and then ultrasonic treatment for 20 min. They were injected (20 mL/min) into the 300 mL distilled water with stirring (600 rpm, 20 min). The rotary evaporation was performed (40 °C, −0.1 MPa) to remove ethanol. Hyaluronan-Cur (20: 5) and zein-Cur (100: 5) nanoparticles were prepared as control group.

Entrapment efficiency (EE) and loading capacity (LC) of Cur were estimated by the reported method of Dai et al. (2018). The amount of Cur was determined at the wavelength of 426 nm using a UV-1800 spectrophotometer. EE and LC were calculated using the following equations (Dai et al., 2018):

$$EE = \frac{\text{Encapsulated curcumin}}{\text{Total curcumin}} \times 100\% \quad (1)$$

$$LC = \frac{\text{Encapsulated curcumin}}{\text{Total mass of nanoparticles}} \times 100\% \quad (2)$$

2.4.2. Light degradation kinetics

The light degradation kinetics of Cur in zein-Cur-hyaluronan composite nanoparticles was estimated refer to the previous literature (Liu, Liu, Zhu, Gan, & Le, 2015). Under the light condition (35 °C, 0.35 W/m^2), sampling time was set at 10, 20, 30, 40, 60 and 90 min. The amount of Cur was measured as the method in Section 2.4.1. In general, the degradation of polyphenols followed the first-order kinetics, and its rate constant (k) and half-life ($t_{1/2}$) were calculated by the following equations (Mercali, Gurak, Schmitz, & Marczak, 2015):

$$Ln(C/C_0) = -kt \quad (3)$$

$$t_{1/2} = \frac{Ln2}{k} \quad (4)$$

Where C and C_0 were the concentration of Cur at time t (h) and the initial.

2.4.3. Simulated gastrointestinal digestion (SGD)

SGD was carried out by the method of Donsì, Voudouris, Veen, and Velikov, (2017). The samples (30 mL) of zein-Cur, LHA-Cur, and zein-Cur-LHA were mixed with the equivalent volume of simulated gastric fluid (SGF, main components: pepsin 3.2 mg/ml, NaCl 2.0 mg/ml, pH 1.2) in a glass flasker. Then the mixed dispersions were placed in a shaking water bath (100 rpm, 37.0 °C, 60 min), and collected samples at 30 and 60 min for analysis. Adjusted pH to 7.5 using 1.0 mol/L NaOH to stop gastric digestion. After that, 30 mL gastric digestion mixtures were mixed with the equivalent volume of simulated intestinal fluid (SIF, main components: pancreatin 2.0 mg/mL, bile salts 12.0 mg/mL, NaCl 8.8 mg/mL, and KH_2PO_4 6.8 mg/mL) aqueous solutions. Further digestion in a shaking water bath (37 °C, 100 rpm), and collected samples at 30, 60, 90 and 120 min for analysis. The collected samples of different digestion stages were centrifuged (100,000 g, 5 min), and Cur was quantified refer to the method in Section 2.4.1.