A Novel Ca-Alginate Nanogel Mediated by Glycyrrhizic Acid

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Abstract. A novel glycyrrhizic acid (GA) mediated Calcium-alginate nanogel was successfully prepared by using GA, calcium chloride ($CaCl_2$) and sodium alginate (SA) through reverse W/O microemulsion combined with controlled internal gelation method. Nanogels were characterized by Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), differential scanning calorimetry (DSC). The mean particle size of GA-Ca-alginate nanogels was 42.54 nm, and the polydispersity index (PdI) was 0.154. Furthermore, GA-Ca-alginate nanogels possessed charged negatively surface with a zeta potential of -44.7 mV. The biocompatibility of GA-Ca-alginate nanogels indicated that no toxicity was found in HepG2 cells. The novel nanogels prepared here have obvious advantages of smaller particle size and may be potential for drug delivery applications.

Introduction

Nanoparticles could be used as effective antitumor drug delivery carriers which can not only improve drug solubility, bioavailability and pharmacological activity, but also protect drug from degradation by kinds of enzymes *in vivo*. Besides, drug-loaded nanoparticles can effectively extend the action time than free drug [1].

Among various controlled drug delivery carrier materials, sodium alginate (SA) is an anionic copolymer consists of (1, 4)-linked β-D mannuronic acid (M units) and α-L-guluronic acid (G units) residues. It is a FDA-approved polymer, with the advantage of non-toxic, mucoadhesive, biocompatible, biodegradable, low in cost, and has been extensively used for kinds of pharmaceutical and biomedical applications [2]. Divalent cations can cooperatively exchange with Na^+ from G groups of alginate to form ionic bridges, and finally form so called “egg-box” structure [3]. There are kinds of methods for preparation of alginate nanoparticles, most of reported methods produce particles with diameters >200 nm [4]. The tumor of liver will filter particles which are less than 10 nm or more than 100 nm, so the nanoparticles should be in the range of 10-100 nm in size [5]. In this work, novel Ca-alginate nanogels modified by GA were prepared with mean particle size under 100 nm by involving Ca^{2+} and GA induced controlled gelification method. We aimed to propose novel target drug delivery carriers, GA-Ca-alginate nanogels, due to their biocompatible and their smaller diameters (<100 nm) which can be uptake by tumor cells

Experimental Section

Prepare of GA-Ca-alginate nanogels. Reverse microemulsion/internal gelation method was used to prepare nanogels. Briefly, 0.5 mL Span 80 and 0.15 mL Tween 80 were dissolved into a 250 mL flask pre-filled with 50 mL paraffin oil act as the oil phase. Then, 15 mL of 0.5% SA solution was added dropwise into the flask to form W/O emulsion by stirring at the speed of 1000 rpm, placing in a water bath at 40 °C. Various phase appearance from transparency to turbidity was obtained during the process of adding SA solution. After emulsification for 1 h, 5 mL of 0.1% (w/v) $CaCl_2$ solution (containing 1.5% GA solution in 60% ethanol solvent) was slowly injected into the W/O nanoemulsion. Subsequently, the emulsion was stirred at 1000 rpm and 40 °C for 1 h to allow

gelation. Nanogels were finally collected by centrifugation. After pre-freezen at -80 °C for 12 h, nanogels were placed in a vacuum lyophilizer and freeze-dried at -80 °C for 24 h to obtain lyophilized nanogels.

Particle size and zeta potential analysis. The GA-Ca-alginate nanogels were dispersed in water and characterized for particle size and zeta potential using dynamic light scattering with a Zetasizer Nano Analyzer (Malvern Instruments Ltd., Malvern, UK).

DSC analysis of nanogels. DSC analysis of the samples was undertaken using a DSC-7 Differential Scanning Calorimetry analyzer (Perkin ELMER, USA), about 6 mg of the sample was weighed into the sample pool to be heated from 30 to 350°C at a speed of 10 °C/min under nitrogen purge at 20 mL /min.

FTIR analysis of nanogels. The sample of the lyophilized nanogels were mixed with KBr and pressed into tablet form. This was then placed in the sample slot of a FT-IR-420 FTIR spectrometer (JASCO, Japan).

Powder X-ray diffraction (XRD) measurement of nanogels. The XRD patterns were used to confirm the crystal phase of the nanogels, The samples were examined in the range of $10° < 2\theta < 50°$.

In vitro Cytotoxicity of GA-Ca-alginate nanogels. The cytotoxicity of the GA-Ca-alginate nanogels on a human hepatoma cell line (HepG2) was measured by using MTT assay. The exponential growth-phase cells were seeded into 96-well plates at 100 µL/well (containing 5000 cells) and cultured for 24 h. The GA-Ca-alginate nanogels were added to wells at different concentrations of GA, and each concentration repeated six times. After cells were incubated for 48 h, 10 µL of MTT was added to each well. After incubated for another 4 h, supernatants were removed and 100 µL dimethylsulfoxide (DMSO) was added to each well. Cell viability was measured by a FlaxStation 3 plate reader (MD, USA) at a wavelength of 570 nm.

Results and discussion

Particle size and zeta potential. The results of mean particle size, polydispersity index (PdI) and zeta potential were shown in Table 1.

DSC analyses of nanogels. In order to investigate the possible physical and chemical interactions between $CaCl_2$, GA and alginate, DSC thermograms of samples were recorded in Fig.1. The DSC thermogram of SA exhibited an exothermic peak at 253°C. Thermogram of SA/$CaCl_2$/GA physical mixture peaks resulted from individual contribution of $CaCl_2$, GA and SA, respectively. In GA-Ca-alginate nanogels, The exothermic peak of SA at 246 °C shifted to 253 °C and the intensity got weaker, which may be because that the interaction between Ca^{2+} and SA, is greater than Na^{+} and alginate macromolecule chain, improving the thermal stability of the formation of GA-Ca-alginate nanogels .

Table 1. Z-Average and Zeta potential of GA-Ca-alginate nanogels

Sample	Z-Average (d.nm)	PdI	Zeta Potential (mV)
GA-Ca-alginate nanogels	42.54	0.154	-44.7

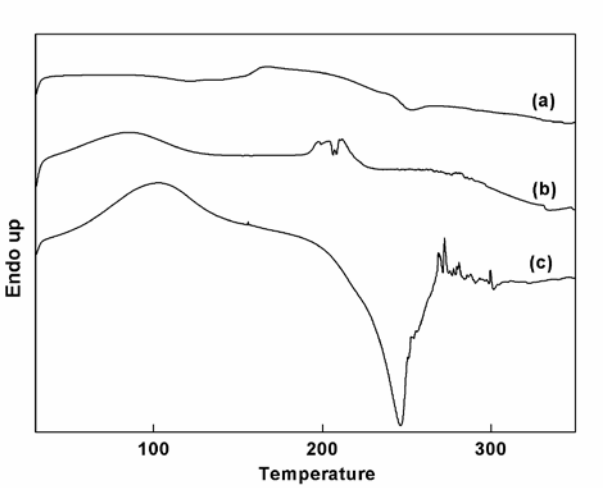

Fig. 1. Thermograms of (a) GA-Ca-alginate nanogels, (b) glycyrrhizic acid, (c) sodium alginate

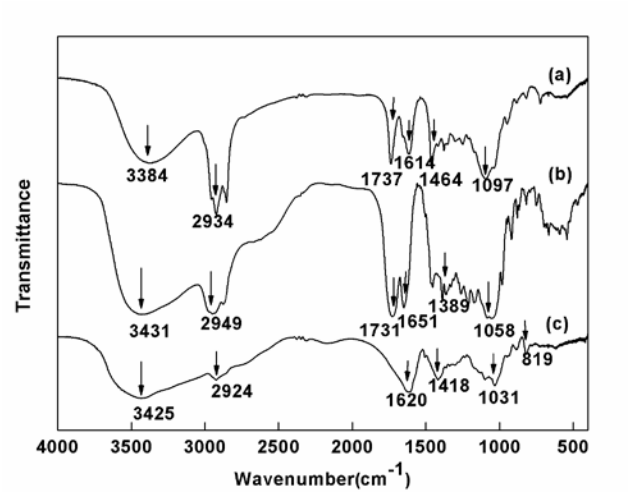

Fig. 2. FTIR spectra of solid (a) GA-Ca-alginate nanogels, (b) glycyrrhizic acid, (c) sodium alginate

FTIR analyses of nanogels. In order to further examine the interaction between components of GA-Ca-alginate nanogels, the spectra of samples were showed in Fig.2. In the spectra of GA-Ca-alginate nanogels, it was showed that the -OH peak of SA and GA was shifted and appeared in the lower wavenumber, which may be due to the interaction between Ca^{2+} and the hydroxyl group of SA, the hydroxyl in the polymer chain of GA also may be the exchange of Ca^{2+} and Na^{+} ion effect. The characteristic absorption peak of SA in 1620, 1418 and 1031 cm^{-1} and GA in 1731, 1651 and 1058 cm^{-1} were shifted to 1617, 1458 and 1101 cm^{-1} respectively, indicating that the COO^{-} of alginate and GA reacted with Ca^{2+}, besides, the absorption peaks of SA at 819 cm^{-1} became weaker and the new characteristic absorption peak emerged at 806 cm^{-1} in GA-Ca-alginate nanogels, indicating that the -OH was involved in the coordination, reduced the bond energy of O-H.

Powder X-ray diffraction (XRD) Spectra of nanogels. The XRD spectra were shown in Fig. 3. The crystal diffraction peak of GA disappeared in GA-Ca-alginate nanogels. It indicated that the GA based amorphous structure dispersed in the nanogels and GA was involved in carrier.

***In vitro* cytotoxicity of GA-Ca-SA nanogels.** The *in vitro* cytotoxicity of the GA-Ca-alginate nanogels was evaluated in HepG2 cells. Fig. 4 showed that the cell viability of GA-Ca-alginate nanogels are about 90% within all the testing concentration, meaning that the GA-Ca-alginate nanogels have no toxicity on HepG2 cells in the given concentration range. It can be used as an efficient drug delivery vehicles because of the good biocompatibility.

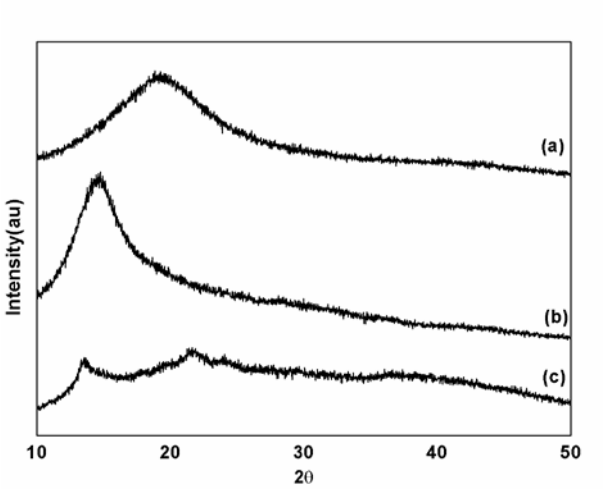

Fig. 3. XRD spectra of (a) GA-Ca-alginate nanogels, (b) glycyrrhizic acid, (c) sodium alginate

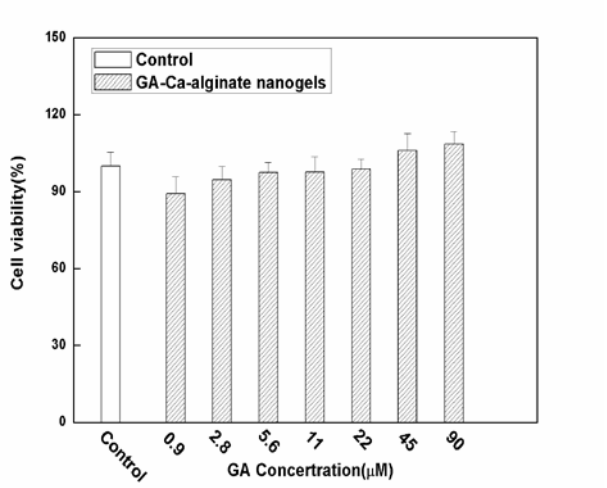

Fig. 4. *In vitro* cytotoxicity of GA-Ca-alginate nanogels

Conclusion

In this study, GA-Ca-alginate nanogels prepared by reverse microemulsion/internal gelation were characterized by small size, storage stability, and good biocompatibility. The narrow particle size distribution with an average particle size of 42.54 nm optimized the minimum size of nanoparticles which has been reported. GA-Ca-alginate nanogels can be used as drug delivery carriers and it will have wide applications for hepatic target therapy.

Acknowledgement

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