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Multilayer sodium alginate beads with porous core containing chitosan based nanoparticles for oral delivery of anticancer drug

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ABSTRACT

To develop efficient and safe anticancer drug doxorubicin hydrochloride (DOX) delivery system for oral chemotherapy, chitosan based nanoparticles (CS/CMCS–NPs) composed of chitosan (CS) and o-carboxymeymethy chitosan (CMCS) were immobilized in multilayer sodium alginate beads (NPs–M–Beads). Two kinds of NPs–M–Beads, with or without porous core, were respectively prepared by internal or external ionic gelation method. In the small intestine, the intact CS/CMCS–NPs were able to escape from porous-beads and sustained release the loading DOX. In vivo results showed that the DOX could be efficiently absorbed by small intestine of SD rat and the higher concentration of the DOX in major organs of rats were found after oral administration of Porous-Beads, which were about 2–4 folds higher than that of non-porous-beads. These results suggested that the NPs–M–Beads with porous core to be exciting and promising for oral delivery of DOX.

1. Introduction

Oral delivery of anticancer drug is still an essential procedures for cancer treatment [1]. Although, it is effective for cancer therapy, the side effect due to the impulse high concentration anticancer-drug in bloodstream and the inconveniences during chemotherapy compromise its clinical treatment efficacy [2]. Oral administration of anticancer drug is a viable alternative to intravenous injection, since it can maintain an optimum blood drug concentration and improves convenience and compliance of patients [3,4]. Nevertheless, anticancer drugs especially those with excellent anticancer effects such as doxorubicin hydrochloride (DOX) and Taxanes (paclitaxel and docetaxel) are not orally bioavailable owing to their peculiar physicochemical properties, and physiological barriers in gastrointestinal (GI) tract [5]. The high cost of manufacturing novel formulations and limited therapeutic window of existing anticancer drugs also restricts the developability for oral route of administration [6].

To improve oral bioavailability of conventional anticancer drugs, nanoparticles immobilized multilayer sodium alginate beads (NPs–M–Beads) were successfully developed by dropping aqueous chitosan/carboxymethyl chitosan nanoparticles blended with alginate (ALG) into $CaCl_2$ solution in our recent study [7]. The crosslinking between $-COO^-$ groups on ALG and Ca^{2+} occurred from external of ALG matrix to form the compact core of multilayer bead. The ALG matrix could protects encapsulated DOX loaded chitosan/carboxymethyl chitosan NPs (DOX:CS/CMCS–NPs) from undesirable drug release in gastric juices [8,9] and rapidly releases the intact DOX:CS/CMCS–NPs in small intestine. However, the limited stability of NPs–M–Beads in small intestine resulting in overly rapid drug release in small intestine compromised oral bioavailability of anticancer drug.

To overcome above limitation, we constructed the porous core of multilayer beads by internal gelation method to improve their ability of drug control release. By dropping aqueous chitosan/carboxymethyl chitosan nanoparticles blended with alginate (ALG) and $CaCO_3$ into hydrochloric acid, the crosslinking between Ca^{2+} and $-COO^-$ groups on ALG occurred from interior of ALG matrix to form a compact core with porous structure in multilayer bead. The crosslinking effect was strengthened in core of porous multilayer beads and it may overcome undesirable drug release encountered in NPs–M–Beads to prolong the contact time

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between formulation and small intestinal mucosa, thereby potentially enhancing drug delivery efficacy [10,11].

In this study, we prepared NPs–M–ALG–beads with porous core to improve their stability in GI tract. The morphology of the NPs–M–ALG–Beads were characterized by scanning electron microscopy (SEM). The drug Loading Efficiency (LE) was determined using a UV–Vis spectrophotometer. The swelling property and release profiles of DOX were evaluated in simulated gastric and intestinal fluid. Finally, the tissue distribution of anticancer drug after oral administration of NPs–M–ALG–Beads was studied qualitatively and quantificationally.

2. Materials and methods

2.1. Materials

CS (molecular weight, MW:10 kDa, degree of deacetylation, DD:89%) was obtained from Biotech Co. (Mokpo, Korea). CMCS (MW:12 kDa, DD:81%, Degree of substitution, DS:92%) was synthesized and characterized by the method described by Chen [12]. Sodium alginate, calcium chloride, liquid paraffin, Span 80 and Tween 80 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium triphosphate (TPP) were purchased from Sigma (St. Louis, USA). DOX was supplied by Zhejiang Hai zheng Co., Ltd. (China). All other reagents and solvents were of analytical grade.

2.2. Preparation of DOX-loaded CS/CMCS–NPs

DOX-loaded CS/CMCS–NPs (DOX:CS/CMCS–NPs) were prepared according to a modified process by Feng [13] (Scheme 1). Briefly, DOX aqueous solution (1 mg/mL, 1 mL) was premixed with CS (1 mg/mL, 6 mL) under magnetic stirring for 15 min. Subsequently, CMCS solution (1 mg/mL, 2 mL) and TPP (1 mg/mL, 1 mL) were blended with the mixture under constant stirring for 30 min, and DOX:CS/CMCS–NPs were formed.

2.3. Preparation of nanoparticles immobilized multilayer sodium alginate beads

Nanoparticles immobilized multilayer sodium alginate beads (NPs–M–ALG–Beads) were prepared by external ionic gelation and Layer-by-Layer (LBL) method, according to Feng [7], which was divided into two processes (Scheme 1). Before the tiny hole dropping process, 5 mL of DOX:CS/CMCS–NPs (1 mg/mL) was sonicated for 5 min, Power 30%. Then, DOX:CS/CMCS–NPs were premixed with 5 mL sodium alginate solution (ALG, 3% w/v) under magnetic stirring for 15 min. Then, the mixture solution was dropped into 100 mL $CaCl_2$ solution (3%, w/v), used an injection syringe without a needle at a dropping rate of 1.0 mL/min and under stirring (100 rpm) for 30 min to form the core of bead. The prepared core beads were removed from the solution and washed the residual $CaCl_2$ with distilled water. During the coating process, the core beads were immersed in ALG (1.5% w/v) solution for 1 min. Then the prepared core beads was poured into liquid paraffin (oil phase) contained span 80 (0.5 mL) and Tween 80 (0.5 mL) in advance while stirring (300 rpm). The $CaCl_2$ solution was then added to the mixture 10 min later and stirred for another 15 min to form 1-layer NPs–M–ALG–Beads. Then the 1-layer beads were taken out from the mixture and rinsed with distilled water until the liquid paraffin was removed completely. The coating process was repeated 3 times until the 3-layer NPs–M–ALG–Beads were prepared.

NPs–M–ALG–Beads with porous core was prepared by internal ionic gelation, whose tiny hole dropping process was different from that of NPs–M–ALG–Beads without porous (Scheme 1). In the tiny hole dropping process, DOX:CS/CMCS–NPs were premixed with 5 mL sodium alginate solution (ALG, 3% w/v) containing 0.5 g $CaCO_3$. Then, the mixture solution was dropped into 100 mL hydrochloric acid (HCl, 0.185% w/v), used an injection syringe without a needle at a dropping rate of 1.0 mL/min and under stirring (100 rpm) for 30 min until the core beads were all floating on the liquid level. The coating process was same as above mentioned.

The prepared NPs–M–ALG–Beads with or without porous core (Porous-Beads, Non-Porous-Beads) were then freeze-dried for 24 h and stored in glass vial.

2.4. Characterization of NPs–M–ALG–Beads

The morphology of the NPs–M–ALG–Beads including porous-Beads and Non-Porous-Beads were observed by scanning electron microscopy (SEM, JSM-6010LA, JEOL Ltd., Japan).

The particle size of NPs–M–ALG–Beads was determined to calculate the average diameter. The mean of diameter (D) was calculated using Eq. (1):

$$D = \sum \mathrm{nd}/\mathrm{n}n \geq 100 \quad (1)$$

where d is the diameter of each beads, n is the total number of beads measured, and D is the mean diameter.

To measure Loading Efficiency (LE) of DOX in beads, the free DOX in supernatants was determined using UV–vis spectrophotometer measurement. In the end of tiny hole dropping process, the Porous-Beads supernatant was assayed spectrophotometrically for DOX content at the wavelength of 480 nm and compared with the standard curve constructed. Supernatant from the Non-Porous-Beads was taken as control sample. All samples were analyzed in triplicate. The LE was determined according to Eq. (2):

$$\mathrm{LE}(\%) = (\mathrm{Dose}_{\mathrm{added}} - \mathrm{Does}_{\mathrm{free}})/\mathrm{Dose}_{\mathrm{added}} \times 100\% \quad (2)$$

where $\mathrm{Dose}_{\mathrm{added}}$ is total amount of DOX added, $\mathrm{Does}_{\mathrm{free}}$ is free DOX in supernatant and W is the weight of freeze-dried beads.

2.5. Swelling study

The swelling characteristic of beads were carried out in four simulated fluids, simulating the complete gastrointestinal (GI) tract environment [14,15]: simulated gastric fluid at pH 1.2 for 2 h, simulated duodenum fluid at pH 6.0 for 2 h, simulated jejunum fluid at pH 7.0 for 2 h, and simulated ileum fluid at pH 7.4 for 2 h. The ingredients of simulated fluids were as follows:

Simulated gastric fluid (SGF, pH 1.2) was prepared by dissolving 0.2 g NaCl, 7 mL concentrated HCl, and 3.2 g pesin in 1 L deionized water; pH was adjusted to 1.2 ± 0.5. Simulated intestinal fluid (SIF, pH 7.4) was prepared by dissolving 6.8 g KH_2PO_4, 190 mL NaOH (0.2 N), and 10.0 g pancreatin in 1 L deionized water; pH was adjusted to 7.4 ± 0.5. Simulated duodenum fluid (pH 6.0) was prepared by mixing SGF pH 1.2 and SIF pH 7.4 in a volume ratio of 30:70; pH was adjusted to 6.0 ± 0.5. Simulated jejunum fluid (pH 7.0) was SIF adjusted to 7.0 ± 0.1. Simulated ileum fluid was SIF adjusted to 7.4 ± 0.1.

The freeze-dried beads were dipped in the corresponding swelling medium with the pH range from 1.2 to 7.4 at 37 °C and shaken (100 rpm) for 8 h. The beads were periodically removed (every 30 min) and weighed till the beads showed constant weight or complete disintegration. The swollen beads were taken out using a blunt-ended forcep and weighed after carefully wiping off residual liquid with a filter paper [16].

All experiments were done in triplicate. The dynamic weight change of the beads was calculated according to Eq. (3):

$$S_W = (W_S - W_0)/W_0 \times 100\% \quad (3)$$