The Alginate Layer for Improving Doxorubicin Release and Radiolabeling Stability of Chitosan Hydrogels

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Abstract

Purpose Chitosan hydrogels (CSH) formed through ionic interaction with an anionic molecule are suitable as a drug carrier and a tissue engineering scaffold. However, the initial burst release of drugs from the CSH due to rapid swelling after immersing in a biofluid limits their wide application as a drug delivery carrier. In this study, alginate layering on the surface of the doxorubicin (Dox)-loaded and I-131-labeled CSH (DI-CSH) was performed. The effect of the alginate layering on drug release behavior and radiolabeling stability was investigated.

Methods Chitosan was chemically modified using a chelator for I-131 labeling. After labeling of I-131 and mixing of Dox, the chitosan solution was dropped into tripolyphosphate (TPP) solution using an electrospinning system to prepare spherical microhydrogels. The DI-CSH were immersed into alginate solution for 30 min to form the crosslinking layer on their surface. The formation of alginate layer on the DI-CSH was confirmed by Fourier transform infrared spectroscopy (FT-IR) and zeta potential analysis. In order to investigate the effect of alginate layer, studies of in vitro Dox release from the hydrogels were performed in phosphate buffered saline (PBS, pH 7.4) at 37 °C for 12 days. The radiolabeling stability of the hydrogels was evaluated using ITLC under different experimental condition (human serum, normal saline, and PBS) at 37 °C for 12 days.

Results Formatting the alginate-crosslinked layer on the CSH surface did not change the spherical morphology and the mean diameter ($150 \pm 10 \mu\text{m}$). FT-IR spectra and zeta potential values indicate that alginate layer was formed successfully on the surface of the DI-CSH. In in vitro Dox release studies, the total percentage of the released Dox from the DI-CSH for 12 days were 60.9 ± 0.8 , 67.3 ± 1.4 , and 71.8 ± 2.5 % for 0.25, 0.50, and 1.00 mg Dox used to load into the hydrogels, respectively. On the other hand, after formatting alginate layer, the percentage of the released Dox for 12 days was decreased to 47.6 ± 1.4 , 51.1 ± 1.4 , and 57.5 ± 1.6 % for 0.25, 0.50, and 1.00 mg Dox used, respectively. The radiolabeling stability of DI-CSH in human serum was improved by alginate layer.

Conclusions The formation of alginate layer on the surface of the DI-CSH is useful for improving the drug release behavior and radiolabeling stability.

Keywords Chitosan hydrogels · Electrospinning system · Doxorubicin release · Alginate · Radiolabeling stability

Introduction

Hydrogels are soft materials of three-dimensional networks formed with various hydrophilic polymers. They can be obtained from many natural hydrophilic polymers, including cellulose, gelatin, dextran, chitosan, alginate, pectin, and hyaluronic acid [1–4]. Although hydrogels are not soluble in water, they can contain a lot of water and retain other guest molecules, such as therapeutic chemical drugs, proteins, and peptides, inside the networks [5]. Because of these properties, hydrogels are widely used as drug delivery carriers, wound dressing sheets, surgical tissue adhesion barriers, and tissue engineering scaffolds [6]. In particular, due to the high porosity and hydrophilicity, the hydrogels are an excellent candidate as a drug delivery carrier. Among these polymers, chitosan is

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one of the most widely used polymers as a drug carrier of hydrogels [7–9].

Chitosan is a derivative of chitin obtained from the shell of crustaceans such as crabs and shrimps. Chitosan has been extensively used in biomedical fields including drug delivery, gene therapy, and regenerative tissue engineering because of its excellent biodegradation, high biocompatibility, low toxicity, as well as facile functionalization with various molecules [10]. In particular, chitosan can readily form electrostatic crosslinked hydrogels with polyanionic molecules due to its cationic property. The electrostatic hydrogels formed under mild conditions can be used to deliver drugs, including enzymes and even cells, which is in contrast with the relatively high toxicity of the hydrogels obtained from the irreversible chemical covalent crosslinking [11]. However, the mechanical properties of the electrostatic hydrogels are not good enough in terms of biomedical fields for which mechanical strength is required. For example, one of the main limitations of hydrogels in applications for tissue engineering is their poor mechanical properties [12, 13]. Furthermore, drug release from the hydrogels can be quick-acting as the drug's diffusion rate through the network structure of the hydrogels [14]. Therefore, a strategy to reinforce mechanical strength and control drug release from the hydrogels is needed. Recently, we reported the doxorubicin (Dox)-loaded and I-131-labeled chitosan hydrogels (DI-CSH) as a selective internal radiochemoembolization agent for cancer therapy [15]. In the previous study, when the CSH were formed through electrostatic crosslinking with tripolyphosphate (TPP) by an electrospinning technique, Dox could physically be loaded into the hydrogels. Within 6 h, Dox from the CSH was rapidly released up to 40 % of the total amount of the drug loaded. Considering the development of Dox-loaded CSH into the products, the effective control of Dox release from CSH without the initial burst release is of therapeutic importance. In this study, we studied alginate-coated CSH as a method to control Dox release rate and maintain radiolabeling stability

(Fig. 1). Alginate, a polysaccharide obtained from brown seaweeds, forms ionic hydrogels through electrostatic interaction between its oligoguluronic acid and molecules of opposite charges. The Dox release and the radiolabeling stability of DI-CSH formed with TPP solution using an electrospinning system were investigated after crosslinking with alginate.

Materials and Method

Materials

Chitosan (190 KDa, Degree of deacetylation: 97 %) was obtained from Youngchipharm Co. (Paju, Korea). Sulfo-succinimidyl-3-[4-hydroxyphenyl]propionate (SHPP) was purchased from Thermo Fisher Co. (Waltham, MA, USA). Doxorubicin (Dox), sodium alginate (15–20 cP, 1 % in water) and sodium tripolyphosphate pentabasic (TPP) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Poly-mesh (Nylon type, open size: $100 \pm 5 \mu\text{m}$) was purchased from TEXTOMA Co. (Deagu, Korea). Na^{131}I was purchased from New Korea Industrial Co. (Seoul, Korea). Human serum was obtained from Chonbuk National University Hospital (Jeonju, Korea).

Preparation of the DI-CSH

Chitosan-SHPP conjugate and the DI-CSH were synthesized and prepared as described in our previous paper with some modifications [15, 16]. Chitosan solution (1 g/100 ml) was mixed with sodium borate buffer (0.5 M, pH 7.3, 100 ml) and SHPP (4 mg). After stirring overnight at 4 °C, the reaction solution was dialyzed with a membrane (MWCO: 12 KDa, Spectrum Laboratory Inc., Rancho Dominguez, CA, USA) against 0.2 M boric acid buffer and distilled water for 4 days. The final solution was lyophilized. $\text{NaI-}^{131}\text{I}$ (1 mCi/0.05 ml) in distilled water was added to the chitosan-SHPP conjugate

Fig. 1 Schematic illustration for formulation of DI-CSH and AG-DI-CSH using an electrospinning system

solution (20 mg/ml) in 0.1 M HCl, immediately followed by the addition of chloramine-T solution (0.1 mg/0.05 ml) dissolved in sodium phosphate buffer (50 mM, pH 7.4). A sodium bisulfite solution (0.1 mg/0.05 ml) dissolved in sodium phosphate buffer was added into the reaction solution after incubation for 10 min. The radiolabeling efficiency of I-131-labeled chitosan-SHPP conjugate was evaluated using ITLC-SG strips with saline as a mobile phase. For DI-CSH, 0.25, 0.5, or 1 mg Dox was added to the I-131-labeled chitosan-SHPP solution and then the mixture was dropped into 100 ml of TPP solution (10 % w/v) by using an electrospinning system as previously described [15]. The DI-CSH were washed and collected by a poly-nylon mesh (pore size: $100 \pm 5 \mu\text{m}$). The final DI-CSH were stored in normal saline.

Crosslinking of the DI-CSH with Alginate

The surface of the DI-CSH was crosslinked and doped with alginate that has the opposite charge of chitosan. The DI-CSH were immersed into 5 ml of the alginate solution (0.5 % w/v, pH 5.0) for 30 min under mild stirring. The alginate-crosslinked DI-CSH (AG-DI-CSH) were washed with normal saline using the poly-nylon mesh. The morphology of DI-CSH before and after crosslinking with alginate was observed using a fluorescence microscope (BX-51, Olympus Co., Tokyo, Japan). To investigate the crosslinking of the DI-CSH with alginate, DI-CSH and AG-DI-CSH were examined by Fourier transform infrared spectroscopy (FT-IR, Spectrum GX, Perkin Elmer Inc., Waltham, MA, USA) and zeta potential analysis (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK).

Dox Release and Radiolabeling Stability of DI-CSH or AG-DI-CSH

The Dox loading efficacy was measured according to our previous paper [15]. The DI-CSH or AG-DI-CSH solutions in PBS (0.1 M, pH 7.4) were incubated at 37 °C for 12 days

under mild stirring. At predetermined time, the hydrogels from the solutions were separated by centrifugation at 5000 rpm for 3 min. After 0.1 ml of supernatant was removed, the same amount of fresh PBS was replaced. The amount of Dox in the supernatant was determined using a UV spectrophotometer at 480 nm. To investigate radiolabeling stability, DI-CSH or AG-DI-CSH were immersed into normal saline, PBS, and human serum. After centrifugation, the supernatant was developed on an ITLC strip using saline as a mobile phase. The strip was scanned using a radio TLC scanner (B-AR-2000-1, BIOSCAN, Washington, DC, USA).

Results

Characteristics of DI-CSH and AG-DI-CSH

The photographs of the hydrogels observed using a microscope were presented in Fig. 2. The conjugation of SHPP to chitosan was characterized and confirmed by $^1\text{H-NMR}$ [16]. The shapes of the DI-CSH and AG-DI-CSH were spherical and uniform with small deviations. The mean diameter of the DI-CSH was $150 \pm 10 \mu\text{m}$ and the size was barely changed after crosslinking with alginate. Figure 3 shows the spectra of the DI-CSH and AG-DI-CSH. The spectrum of DI-CSH shows the characteristic absorption bands of chitosan at 3414 (O-H stretching), 2934 (C-H stretching), 1646 (amide I vibration), 1595 (amide II vibration), and 1079 cm^{-1} (C-O stretching), respectively. After crosslinking with alginate, the spectrum shows the following absorption peaks: increased peak at 1600 cm^{-1} (new chemical structure between $-\text{NH}_3^+$ and $-\text{COO}^-$) and strong band at 1410 cm^{-1} (symmetric COO^- stretching). The presence of alginate layer on DI-CSH was investigated by zeta potential analysis. As shown in Fig. 4, DI-CSH were positively charged with +30.6 mV, whereas the zeta potential value of DI-CSH was changed to negative charge of -65.5 mV after crosslinking with alginate.

Fig. 2 The photographs of (a) DI-CSH and (b) AG-DI-CSH obtained by light microscopy ($\times 40$ magnification). The scale bars indicate 200 μm

Fig. 3 FT-IR spectra of DI-CSH (solid line) and AG-DI-CSH (dot line)

Dox Release and Radiolabeling Stability of DI-CSH and AG-DI-CSH

The Dox release properties from DI-CSH or AG-DI-CSH were presented in Fig. 5. In all samples, the percentages of Dox release from the hydrogels were increased for 12 days as the amount of Dox in the DI-CSH was increased. At 12 days, the percentages of Dox release from the DI-CSH were 60.9 ± 0.8 , 67.3 ± 1.4 , and 71.8 ± 2.5 % for 0.25, 0.50, and 1.00 mg

Fig. 5 In vitro doxorubicin release properties from DI-CSH and AG-CSH at different drug loading amount in PBS for 12 days at 37 °C

Dox used to load into the hydrogels, respectively. Crosslinking of alginate on the surface of DI-CSH reduced the release of Dox from the hydrogels. The percentage of Dox release from the AG-DI-CSH were 47.6 ± 1.4 , 51.1 ± 1.4 , and 57.5 ± 1.6 % for 0.25, 0.50, and 1.00 mg Dox used to load into the hydrogels, respectively. The radiolabeling of the DI-CSH in PBS or saline was stable for 12 days, whereas in human serum, the radiolabeling stability of DI-CSH was decreased to 80.9 ± 6.6 %. The radiolabeling stability of DI-CSH in human serum was improved by formatting alginate layer to 92.8 ± 2.5 % (Fig. 6).

Fig. 4 Zeta potential values of (a) DI-CSH and (b) AG-DI-CSH

Discussion

CSH formed with polyanionic molecules such as TPP have high biocompatibility and are often used in various biomedical fields. However, the use of CSH as a drug delivery carrier has limitations due to the weak mechanical properties that contribute to the initial burst drug release from CSH. In this study, we used alginate to improve Dox release properties and radiolabeling stability of DI-CSH. Because carboxyl acid groups of alginate can electrostatically interact with amine groups of chitosan, alginate allows the crosslinked layer on the surface of CSH [17]. The alginate layer formed on the surface of DI-CSH is anticipated to act as a barrier to control drug release rate and to play a role in maintaining the radiolabeling stability.

The interaction between DI-CSH and alginate was evaluated by FT-IR spectroscopy (Fig. 3). From the spectra results, it was found that there were changes in the absorption peaks after crosslinking with alginate. In particular, a strong increase of the peak at 1600 cm^{-1} indicates new chemical structure between $-\text{NH}_3^+$ of chitosan and $-\text{COO}^-$ of alginate

[18]. Moreover, in the case of AG-DI-CSH, the characteristic peak at 1410 cm^{-1} , which was attributed to carboxyl group of alginate, demonstrates the presence of alginate in the hydrogels. The formation of the alginate layer was confirmed by measuring the change of the surface charge of the hydrogels. The change of the zeta potential value of DI-CSH after crosslinking of alginate from positive to negative charge indicates that the alginate layer on the surface of DI-CSH was formed. From the results of the FT-IR and zeta potential, the presence of alginate layer on the surface of the DI-CSH through ionic interaction between amine groups of chitosan and carboxylic acid groups of alginate was confirmed.

The release kinetics of drugs from hydrogels is dependent on the drug diffusion rate and physicochemical properties of the hydrogels [19]. In general, release of drugs dispersed within the three-dimensional structure of the hydrogels occurs through diffusion of the drugs and swelling of the hydrogels [20]. Once the hydrogels including drugs come into contact with a biofluid, the hydrogels may swell or grow as water or fluid is absorbed. The drug then diffuses out of the domain of the hydrogels. With a facile method, the drug release behavior can be controlled through formation of another surface layer on the hydrogels. The initial Dox release rate from the DI-CSH was slower by alginate layer on the hydrogels (Fig. 5). As the amount of Dox dispersed in the DI-CSH is increased, the total amount of the drug release is increased. This result is due to the difference of swelling ratio of the hydrogels and diffusion rate of Dox between the DI-CSH and AG-DI-CSH. Likewise, the radiolabeling stability of DI-CSH was improved by alginate layer on the surface of the hydrogels. The formation of the crosslinked layer of alginate on the surface of the DI-CSH is achieved through an ionic interaction between chitosan and alginate and can improve the drug release behavior, in particular, the initial rapid release rate and radiolabeling stability (Fig. 6).

Fig. 6 The radiolabeling stability of DI-CSH and AG-DI-CSH in PBS for 12 days at 37°C

Conclusion

In this study, in order to improve the drug release behavior and radiolabeling stability of DI-CSH, alginate was used for formatting a layer on the surface of the DI-CSH. The alginate layer formed through ionic interaction between amine groups of chitosan and carboxylic acid groups of alginate was confirmed by FT-IR spectroscopy and zeta potential analysis. By alginate coating, we were able to show that the initial rapid release rate of Dox from DI-CSH is regulated and the radiolabeling efficiency is improved. Therefore, formatting the alginate layer on the surface of the DI-CSH can be a useful strategy for improving their drug release behavior and radiolabeling stability.

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Conflicts of Interest Jeong Il Kwon, Chang-Mon Lee, Hwan-Seok Jeong, Phil-Sun Oh, Hyosook Hwang, Seok Tae Lim, Myung-Hee Sohn, and Hwan-Jeong Jeong declare that they have no conflict of interest.

Ethical Statements The manuscript does not contain human and animal studies or patient data.

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