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Hygroscopicity modulation of hydrogels based on carboxymethyl chitosan/Alginate polyelectrolyte complexes and its application as pH-sensitive delivery system

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ABSTRACT

Polyelectrolyte complex (PECs) hydrogels with high hygroscopicity modulation ability were successfully prepared based on carboxymethyl chitosan (CMCS)/alginate. The swelling ratio of the hydrogels could be tuned from 1 to 450 simply by changing the weight ratio of CMCS to alginate, and superabsorbent hydrogels were obtained when the weight ratio of CMCS to alginate was below 1/1. Also, the swelling kinetics and water diffusion mechanism in the hydrogels were discussed. In vitro cytotoxicity results indicated that hydrogels had excellent cytocompatibility. The swelling ratio of the hydrogel as well as its BSA releasing profile was notably pH dependent. Compared with the values at pH 1.2, the swelling ratio of hydrogels (CMCS/alginate weight ratio of 1/2) at pH 7.4 was about 34 times higher, and the amount of BSA released at pH 7.4 was also significantly higher. The as-prepared CMCS/alginate PECs hydrogels hold great potential for oral delivery of protein drugs through the intestinal tract.

1. Introduction

As either synthetic or natural macromolecules bearing ionizable groups, polyelectrolytes (PEs) can form polyelectrolyte complexes (PECs) by establishing electrostatic interactions with oppositely charged PEs (Lalevee et al., 2016; Michaels, 1965; Saether, Holme, Maurstald, Smidsrod, & Stokke, 2008). PECs prepared from natural biopolymers, especially polysaccharides, without any chemical covalent cross-linkers, are usually considered as non-toxic, well-tolerated, cytocompatible, and have been studied for various biomedical applications in the form of nanoparticles, hydrogels, scaffolds, or coacervates, etc (Conzatti et al., 2017; Kim, Lee, & Kim, 2004; Luo & Wang, 2014).

Chitosan is a cationic charged linear polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine residues, which is produced by deacetylation of chitin, the second most plentiful polymer in nature behind only cellulose (Drury & Mooney, 2003; Rinaudo, 2006). Known for its low toxicity, bioactivity, biodegradability and mucoadhesive properties (Verma, Dubey, Verma, & Nayak, 2017), chitosan has been widely studied to create PECs for various applications by complexing with anionic macromolecules, such as alginate (Conzatti et al., 2017), hyaluronic acid (Lalevee et al., 2016), pectin (Chang & Lin, 2000), carrageenan (Tapia, Corbalan, Costa, Gai, & Yazdani-Pedram, 2005), gum arabic (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007), carboxymethyl cellulose (H. Chen & Fan, 2008), dextran sulfate (Y. Chen, Mohanraj, & Parkin, 2003), xanthan gum (Lal, Dubey, Gaur, Verma, & Verma, 2017) and chondroitin sulfate (Huang, Sui, Wang, & Jiao, 2010), etc.

Due to the great potential of H-bonds formation between their segments, chitosan with large molecular weight is non-soluble in neutral and alkaline water (Rinaudo, 2006). By substituting some amino and hydroxyl sites of its glucosamine units with carboxymethyl groups, carboxymethyl chitosan (CMCS), a water-soluble derivative of chitosan, is formed (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Lin, Liang, Chung, Chen, & Sung, 2005). Besides good water solubility, CMCS has shown excellent biological properties as well as moisture retention ability, film-forming ability and non-toxicity (Hu et al., 2016; Yan et al., 2016). As a natural polyanionic polymer obtained from algal or bacterial sources, alginate is non-toxic and biodegradable, composed of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues arranged either as consecutive blocks or in a random distribution (El-Sherbiny, 2010; Pawar & Edgar, 2012). CMCS/alginate hydrogels, cross-linked by Ca^{2+} (Tavakol, Vasheghani-Farahani, Dolatabadi-Farahani, & Hashemi-Najafabadi, 2009; Zhang, Guo, Peng, & Jin,

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2004), genipin (S.C. Chen et al., 2004) or glutaraldehyde (Mi et al., 2005), have been prepared and evaluated as potential carriers for drug delivery in the form of membranes, beads and microcapsules. However, the use of cross-linking agents such as glutaraldehyde to chemically form polymeric hydrogels may lead to unwanted reactions with drugs or toxic side effects because of the residual cross-linking agents (Lin et al., 2005).

The pK_a values of CMCS's amino-groups is about 6.5, while the pK_a values alginate's carboxyl groups is about 3.5, indicating that PECs will be formed by the ionic interaction between the carboxylate moieties on alginate with the protonated amine groups on chitosan in the pH interval of 4–6 (Lawrie et al., 2007; Mukhopadhyay, Sarkar, Soam, & Kundu, 2013). In this study, ionotropic hydrogels of CMCS/alginate PECs without any cross-linkers were prepared for the first time. The swelling ratios of the hydrogels could be widely tuned in the range of 1–450 simply by changing the weight ratio of CMCS to alginate, and the hydrogels showed pH-responsive ability and were potentially applied in site-specific delivery of drugs to the gastrointestinal tract.

2. Materials and methods

2.1. Materials

Chitosan (weight average molecular weight (M_w) of ~ 620 kDa, degree of deacetylation = 84% determined by acid-base titration), sodium hydroxide (NaOH), isopropyl alcohol, acetic acid (97%), potassium acetate, potassium chloride, absolute methanol, Bovine Serum Albumin (BSA) and absolute ethyl alcohol were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Sodium alginate ($M_w \geq 700 \times 10^4$, M/G ratio 1.5) was purchased from Qingdao Bright Moon Seaweed Group Co., Ltd. Monochloroacetic acid was acquired from Xin Xing Reagent Co., Ltd., Liaoning. All chemicals and reagents were used as received without any further purification. D.I. water with a resistivity of 18.2 MΩ•cm was obtained from a Milli-Q Biocel water purifier, and used throughout these experiments.

2.2. Synthesis and characterization of carboxymethyl chitosan (CMCS)

CMCS was synthesized by a procedure as in the literature with slight modifications (Fernanda & de Abreu, 2005). The preparation of CMCS was confirmed by Fourier Transform Infrared (FTIR) spectroscopy, which was carried out by an Agilent Technologies Cary 630 spectrometer with KBr pellets. Pellet for each measurement was formed by 1 mg of chitosan or CMCS mixed with 0.1 g of KBr, and was scanned from 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1}.

The degree of substitution (DS) and deacetylation (DD) of the prepared CMCS were determined by a potentiometric titration method (Kong, 2012; Liu, Chen, Jin, Sun, & Gao, 2004). Typically, 0.25 g CMCS was dissolved in 40 mL of 0.1 M HCl standardized aqueous solution, which was titrated with 0.1 M NaOH standardized aqueous solution. The volume of NaOH added was recorded and the pH value of the CMCS solution was simultaneously recorded by a pH meter. DS and DD were calculated as follows:

$$DS = \frac{203C(V_2 - V_1)}{m - 80C(V_2 - V_1) + 22C(V_3 - V_0) + 42C(V_3 - V_2)}$$

$$DD = \frac{203C(V_3 - V_2)}{m - 80C(V_2 - V_1) + 22C(V_3 - V_0) + 42C(V_3 - V_2)} \times 100\%$$

Where C is the concentration of NaOH aqueous solution (mol/L). V_1 is the volume of sodium hydroxide consumed by excessive hydrochloric acid (mL), V_2 is the volume of sodium hydroxide for the titration of −COOH terminal, and V_3 is the volume of sodium hydroxide for the titration of $-NH_3^+$ and $-NH_2^+CH_2COO^-$ terminal. V_0 is the volume of sodium hydroxide used for the neutralization of hydrochloric acid in the blank test (mL). m is the weight of the sample (mg).

Table 1
Some swelling and diffusion parameters of the CMCS/alginate hydrogels with distinct CMCS-to-alginate weight ratios in D.I. water.

Sample		Swelling kinetics			Diffusion characteristic
Name	CMCS/ Alginate (*w/w*)	Initial swelling rate, r_i (g/ m/min)	Equilibrium swelling ratio, q_e (g/g)	Swelling rate constant, k_2 (g/g/min)	Swelling exponent, *n*
CA-1	1/2	2.82	558.66	9.04E-06	0.83
CA-2	2/3	1.93	487.80	8.10E-06	0.83
CA-3	4/5	1.10	396.83	6.99E-06	0.83
CA-4	19/20	0.56	273.22	7.45E-06	0.81
CA-5	1/1	0.53	47.42	2.38E-04	0.48
CA-6	3/2	0.31	13.92	1.62E-03	0.37
CA-7	2/1	0.18	2.99	1.97E-02	0.18

2.3. Preparation and characterization of hydrogels based on carboxymethyl chitosan/alginate PECs

CMCS solution was prepared by dissolving 1.5 g of as-prepared CMCS in 100 mL D.I. water, and filtered to remove any undissolved materials. Alginate solution was made by dissolving 0.6 g of alginate in 40 mL D.I. water. CMCS and alginate solution with various weight ratios as in Table 1 were mixed and stirred overnight. After sonication for 1 h to remove trapped air bubbles, the mixtures were poured into glass petri dishes to give a liquid layer and put into the setup as in Scheme 1b. Gels were formed upon exposure to acetic acid atmosphere for 5 h. When a polycation and a polyanion are mixed when fully charged, precipitation will usually occur (Thang Trung, Aarstad, Skjak-Braek, Draget, & Varum, 2013). However, by using the above method, we successfully obtained a homogeneous gel. The gelled layers were washed several times with D.I. water, and air-dried at 50℃ for 24 h to give the corresponding membranes. The membranes were cut into pieces (2 cm × 2 cm) for the swelling behavior studies.

The solution, hydrogels and dried films were studied with a UV–vis spectrometer (T6 New Century) at a wavelength from 400 to 800 nm and DTA (Setsys Evolution 18, SETARAM).

The cytotoxicity of CMCS/alginate hydrogels was evaluated in a 96-well plate using NIH-3T3 fibroblast cells by extraction test and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test according to ISO 10993 part 5 guidelines (ISO document, 1992). Briefly, NIH-3T3 cells were seeded into 96-well plates at a density of 3000 cells per well. After incubation at 37℃ for 24 h in a humidified atmosphere of 5% CO_2, the medium in each well was replaced with the extracts. Normal culture media were used as the control. After further culturing for 48 h, 20 μL of MTT (5 mg/mL in PBS) was added to each well. 4 h later, the medium with MTT was replaced with 150 μL dimethyl sulfoxide (DMSO). After incubation for 15 min with agitation, the absorbance of the DMSO solution at 490 nm was measured by a plate reader (BIO-RAD iMark). Experiments were carried out in four times. The relative cell viability was expressed as the mean ± SD of the calculated absorbance ratio of the extraction treated sample to the control.

2.4. Swelling behavior of CMCS/alginate hydrogels

Swelling ratio of the CMCS/alginate complex hydrogels was studied by the gravimetric method. Test samples of the hydrogels were weighed before and after immersion at 25℃ or 37℃ in 30 ml of D.I. water, HCl solution simulating gastric fluid (SGF, pH 1.2), and a phosphate buffered saline (PBS) solution simulating intestinal fluid (SIF, pH 7.4) (Patel & Amiji, 1996; Risbud, Hardikar, Bhat, & Bhonde, 2000). At regular intervals, the swollen samples were taken out from the medium, and weighed immediately after being blotted with a piece of filter paper to remove any surface water. The measurements were continued until equilibrium swelling state was achieved. The swelling ratio was