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Preparation and properties of novel hydrogel based on chitosan modified by poly(amidoamine) dendrimer

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ABSTRACT

Currently, chitosan (CTS) or chitosan derivatives hydrogels are applied in different fields, such as biological materials, medical materials and hygiene materials. In this study, novel chitosan hydrogels were successfully prepared by chitosan and poly(amidoamine) (PAMAM) dendrimer with glutaraldehyde serving as a cross-linking agent. Fourier transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (^{1}H NMR) and gel permeation chromatography (GPC) were performed to characterize PAMAM. The structure and morphology of hydrogels were characterized by FTIR, thermo gravimetry analysis (TGA), and scanning electron microscopy (SEM). The swelling properties of the hydrogels were investigated in solutions of pH 1.0 and 7.4. The hydrogels showed good swelling capacities and pH-sensitive swelling properties. Besides, the antibacterial activities of the hydrogels against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) were tested by optical density. Compared with the pure chitosan hydrogel, their antibacterial activities were significantly improved with the increase in the blending ratio of PAMAM. And with the increase in cross-linking agent and concentration of CTS, the antibacterial activities increased firstly and then slightly decreased. The hydrogel was expected to be a novel antibacterial material.

1. Introduction

Chitosan is a natural cationic polymer with biocompatibility, non-toxicity, biodegradability, antibacterial activity and antioxidant activity [1–3]. Based on these advantages, chitosan is often used in biological, chemical and medical fields. It is a weak polybase with a pKa around 6.5, making its charge density vary in the different pH range. Reactive amine group ($-NH_2$) and hydroxyl group (–OH) of chitosan per glucosidic unit make it convenient for so many chemical reaction such as graft-polymerization [4–10] of monomers onto it. Furthermore, chitosan is easily cross-linked by glutaraldehyde to make hydrogels, which are pH-sensitive and widely used as wound dressing or other biomedical materials [11–25]. Even if this CTS hydrogel retains some amine groups, its antibacterial activity is limited in practical application. Therefore a new antibacterial agent was introduced into the CTS hydrogel to enhance its antibacterial activity.

Dendrimer is a kind of symmetrical and spherical molecule. In these years, it has attracted more and more attention of the researchers, due to its branching sites and terminal groups that usually form a well-defined surface [26–31]. In addition, because of their bio-friendly nature and unique carrier properties, they show promise to outperform many polymeric materials for medical applications. Some researchers made chitosan-dendrimer hybrid to obtain a variety of good performances [32]. Different from the method above, we used a simple and effective method of directly cross-linking chitosan and dendrimer by glutaraldehyde. Poly(amidoamine) (PAMAM) dendrimer is a good candidate to enhance the antibacterial activity of the hydrogel, which has abundant amine groups compared with other molecules or materials. PAMAM-2.0 generation (2.0G) has good antibacterial activity (effective antimicrobial therapy of local trauma), and does not induce antibiotic resistance in bacteria. Besides, it is non-toxicity and easy synthesized, compared with high generation PAMAM which usually requires complex reaction processes [33–36].

In this study, firstly PAMAM-2.0G was successfully synthesized confirmed by Fourier transform infrared spectroscopy (FTIR), ^{1}H nuclear magnetic resonance (^{1}H NMR) and gel permeation chromatography (GPC). Then the novel chitosan hydrogels modified by PAMAM-2.0G were prepared successfully to improve the antimicrobial properties under the different technological parameters. And their structure and morphology were characterized by FTIR, scanning electron microscopy (SEM) and thermo gravimetry analysis (TGA). The swelling properties were studied in different buffer

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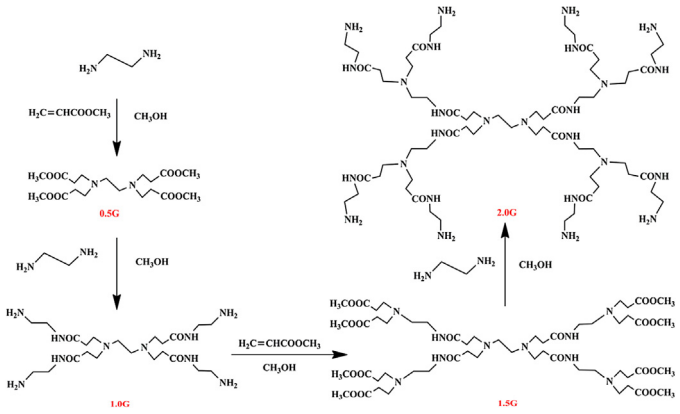

Fig. 1. Synthesis of different generations PAMAM dendrimers.

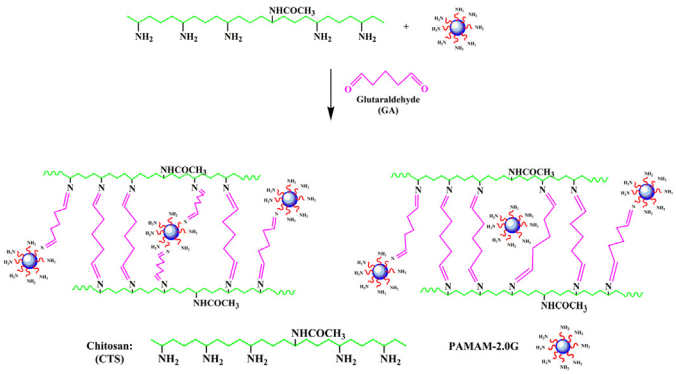

Fig. 2. Preparation of CTS-PAMAM hydrogels.

solutions and antibacterial activities were studied against Gram-negative *Escherichia coil* and Gram-positive *Staphylococcus aureus*. The hydrogel was expected to be a novel antibacterial material and maybe applied in the field of drug carrier.

2. Experimental section

2.1. Materials

Ethylenediamine (EDA) and methyl acrylate (MA) were obtained from Tianjin Fuchen Chemical Reagents Factory. Glutaraldehyde (25%) aqueous solution was obtained from Sinopharm Chemical Reagent Co., Ltd. Chitosan (DD = 91%) was obtained from Zhejiang Golden-Shell Pharmaceutical Co., Ltd. All other chemicals were of analytical grade and were used as received.

2.2. Synthesis of PAMAM dendrimers

PAMAM dendrimers were prepared following the previously reported procedure [34,35]. Briefly, EDA (0.075 mol) was dissolved in 40 mL methanol and added drop wise to a stirred methyl acrylate (MA) (0.6 mol) under nitrogen atmosphere. The mixed solution was stirred at 0 °C for 30 min and kept at 30 °C for 24 h. Then, the methanol and excess methyl acrylate were removed under reduced pressure by a rotary evaporator to get half generation (0.5G) PAMAM.

PAMAM-0.5G (0.025 mol) in 50 mL methanol was added drop wise to EDA (0.6 mol) under nitrogen atmosphere. The final mixture was stirred at 0 °C for 30 min, and the reaction was kept at 30 °C for 24 h. Then the methanol and excess EDA were removed under reduced pressure to get one generation (1.0G) PAMAM. PAMAM-1.5 generation (1.5G) and PAMAM-2.0G were prepared by repeating the above procedures. Fig. 1 is the synthesis of different generation PAMAM dendrimers.

2.3. Preparation of CTS-PAMAM hydrogels

Chitosan was completely dissolved in acetic acid solution. PAMAM-2.0G was dissolved in hydrochloric acid and the pH value was adjusted to 5–6. Two kinds of solutions were mixed for some time, then a certain concentration of glutaraldehyde solution was added drop wise to the mixed solution. After stirring evenly, the mixture was placed at 25 °C for 2 days and chitosan-PAMAM (CTS-PAMAM) hydrogel was formed. The hydrogel was washed with ethanol and freeze-dried to get product. Fig. 2 showed the preparation of CTS-PAMAM hydrogels, and it had two possible interaction mechanisms. Design of different conditions on preparation of CTS-PAMAM hydrogels is shown in Table 1.

2.4. ^1H nuclear magnetic resonance

The ^1H NMR spectrum of PAMAM-2.0G was determined on NMR spectrometer (Varian Co., Ltd. Mercury Plus 400) at 400 MHz using D_2O as a solvent. Chemical shifts were reported in ppm using tetramethylsilane (TMS) as an internal reference.

2.5. Gel permeation chromatography

Gel permeation chromatography (GPC, Water 600E, Waters, USA) was used to characterize the molecular weights of PAMAM-2.0G. The GPC instrument was assembled with the chromatographic column (Ultra-hydrogel 500) and the refractive index detector (Waters 2414). The mobile phase was 0.1 mol/L $NaNO_3$ solution at a flow rate of 1.0 mL/min at 35 °C. Standard curve was carried out by PEG standards. Data analyses were carried out on Breeze 2 HPLC System.

2.6. Fourier transform infrared spectrum

The FTIR spectra of PAMAM-0.5G, 1.0G, 2.0G, CTS, CTS hydrogel and CTS-PAMAM hydrogel were characterized by FTIR spectroscopy (Lambda 750 S, Perkin Elmer Instruments Co., Ltd.). CTS hydrogel and CTS-PAMAM hydrogel were the freeze-dried samples. The scanning wavenumber range was 4000–400 cm^{-1}.

Table 1
Different conditions on preparation of CTS-PAMAM hydrogels.

	2.0G/(2.0G + CTS) (wt %)	−CHO: $-NH_2$ of CTS (molar ratio)	Concentration of CTS (wt %)
variation of PAMAM content	0	0.7	3.0
	10	0.7	3.0
	20	0.7	3.0
	30	0.7	3.0
	40	0.7	3.0
variation of cross-linking agent dosage	30	0.5	3.0
	30	0.7	3.0
	30	0.9	3.0
	30	1.1	3.0
	30	1.3	3.0
variation of chitosan concentration	30	0.7	2.0
	30	0.7	2.5
	30	0.7	3.0
	30	0.7	3.5
	30	0.7	4.0