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Degradation and compatibility behaviors of poly(glycolic acid) grafted chitosan

Luzhong Zhang [a], Sufeng Dou [a], Yan Li [b], Ying Yuan [a], Yawei Ji [a], Yaling Wang [c], Yumin Yang [a,*]

[a] Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong 226001, P. R. China
[b] College of Life Science and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 210009, P. R. China
[c] College of Chemistry and Chemical Engineering, Nantong University, Nantong 226001, P. R. China

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ABSTRACT

The films of poly(glycolic acid) grafted chitosan were prepared without using a catalyst to improve the degradable property of chitosan. The films were characterized by Fourier transform-infrared spectroscopy and X-ray photoelectron spectroscopy (XPS). The degradation of the poly(glycolic acid) grafted chitosan films were investigated in the lysozyme solution. *In vitro* degradation tests revealed that the degradation rate of poly(glycolic acid) grafted chitosan films increased dramatically compared with chitosan. The degradation rate of poly(glycolic acid) grafted chitosan films gradually increased with the increasing of the molar ratio of glycolic acid to chitosan. Additionally, the poly(glycolic acid) grafted chitosan films have good biocompatibility, as demonstrated by *in vitro* cytotoxicity of the extraction fluids. The biocompatible and biodegradable poly(glycolic acid) grafted chitosan would be an effective material with controllable degradation rate to meet the diverse needs in biomedical fields.

1. Introduction

Chitosan is a linear natural polysaccharide containing β-(1,4)-2-amino-D-glucose and β-(1,4)-2-acetamindo-D-glucose unit, and obtained by N-deacetylation of chitin extracted from crustacean shells [1]. Because of its good biocompatible, antibacterial and low toxic properties, chitosan has been widely used for biomedical applications, such as sustained drug and protein delivery [2–4], non-viral gene delivery [5] and tissue engineering [6,7]. However, the use of chitosan in the biomedical fields has been limited because of the slow degradation rate [8], poor mechanical properties and the fact that it is hard to handle [9]. Since grafting copolymerization can modify the structure and properties of natural polysaccharides, it is an important resource for developing advanced materials [10,11]. Chitosan has reactive amine and hydroxyl side groups occurring in the units of polymer chains, and it acts as a desirable backbone to graft synthetic polymer. For instance, chitosan-g-N-isopropylacrylamide copolymer with thermoresponsive, fully reversible property was synthesized [12]. In the view of the designing of biologically degradable tissue engineering biomaterials, it would be advantageous to prepare a grafting copolymer composed of chitosan and poly(glycolic acid).

Poly(glycolic acid), which is a fascinating synthetic polymer, has exhibited good mechanical properties, biodegradability and biocompatibility [13]. It has been widely used in biomedical applications, such as tissue engineered scaffold [14,15], controlled drug delivery systems [16] and implants for orthopedic device [17]. However, poly(glycolic acid) generally produces acidic degradation products at the implanted site. The degraded glycolic acid decreases the pH in the surrounding of the scaffold, and then evokes inflammatory tissue reactions [18]. Since basic glucosamine released from degrading chitosan is expected to neutralize acidic products, the tissue reactions may be alleviated by chitosan grafting copolymer. Importantly, poly(glycolic acid) grafted chitosan could not only retain the good properties of chitosan, but also may bring the easy degradation.

In the present work, the films of poly(glycolic acid) grafted chitosan were prepared without using a catalyst to improve the degradable properties of chitosan. The films were characterized by Fourier transform-infrared spectroscopy (FT-IR) and X-ray photoelectron spectroscopy (XPS) analysis. The degradation rates of the poly(glycolic acid) grafted chitosan films were investigated in the lysozyme solution. It is found that the poly(glycolic acid) grafted chitosan films are more easily degradable compared with chitosan film, and the degradation rate of poly(glycolic acid) grafted chitosan films could be regulated. The *in vitro* cytotoxicity of the extraction fluids revealed that the films of poly(glycolic acid) grafted chitosan have no obvious cytotoxic effect.

2. Experimental section

2.1. Materials and methods

Chitosan (the average molecular weight was 2.8×10^4 Da, the degree of deacetylation was about 95.3%) was purchased from Nantong Xincheng Biochemical Company. The glycolic acid was obtained from Acros Organics Company. The lysozyme was bought from Shanghai

* Corresponding author. Tel./fax: +86 513 85511585.
E-mail address: yangym@ntu.edu.cn (Y. Yang).

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Aladdin Reagent Company. Other reagents were used as received without further purification. FT-IR spectra of chitosan and poly(glycolic acid) grafted chitosan films were recorded with a spectrometer (Nicolet 5700, Madison, WI). The X-ray photoelectron spectroscopy (XPS) of chitosan and poly(glycolic acid) grafted chitosan films were recorded on a Thermo Scientific K-Alpha spectrometer (Thermo Scientific, USA) equipped with a monochromatic Al-K_α X-ray source. The morphology of the chitosan film and the poly(glycolic acid) grafted chitosan films before and after degradation were observed using an S-3400 NII scanning electron microscopy (SEM, Hitachi, Japan). The samples were coated with gold using a JFC-1600 unit (JEOL Inc., Japan) Ion Sputter before examination under the SEM.

2.2. Preparation of poly(glycolic acid) grafted chitosan films

Chitosan was dissolved in glycolic acid solutions with different concentrations and the mixtures were then standing overnight at room temperature. The solutions were poured into frame molds and dried at 45 °C to a constant weight. Then, the films were treated at 80 °C at a reduced pressure (10–12 mm Hg) for another 24 h to promote dehydration of the copolymer salts and the polymerization of glycolic acid. At last, the samples were extracted with methanol to remove unreacted glycolic acid and oligo(glycolic acid).

2.3. Preparation of chitosan film

Chitosan was dissolved in acetic acid solutions with different concentrations and the mixtures were then standing overnight at room temperature. The solution was poured into a frame mold and dried at 45 °C to a constant weight. The samples were then extracted with methanol to remove acetic acid, and then the films were immersed in 2 M sodium hydroxide solution for 4 h. All prepared films were sterilized with 70% alcohol and washed with sterilized phosphate buffered saline (PBS, 0.1 M, pH 7.4) prior to use.

2.4. In vitro degradation of poly(glycolic acid) grafted chitosan films and chitosan film

The enzymatic degradation of poly(glycolic acid) grafted chitosan films *in vitro* was investigated in the lysozyme solution. The poly(glycolic acid) grafted chitosan films (0.1 g) were immersed in 10 mL of lysozyme solution (2 mg/mL) in phosphate buffered saline (PBS) (pH 7.4) at 37 °C. For the sake of comparison, chitosan film was treated in the same manner. All the solutions were changed weekly, and the films were taken out at the predetermined time points. The degraded samples were washed three times with distilled water, dried under vacuum at 40 °C and weighted. The measurements were performed in triplicate and the results were the average of the three times. The degradation ratio was determined according to the following equation:

$$\text{Degradation ratio} = \frac{\text{Initial dry weight} - \text{dry weight after degradation}}{\text{Initial dry weight}}.$$

2.5. Cell culture

The mouse fibroblast cells (L-929) were grown at 37 °C in a humidified atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM). The culture medium was supplemented with 10% fetal calf serum, 100 μg/mL streptomycin, 100 U/mL penicillin and 4×10^{-3} M L-glutamine.

2.6. In vitro cytotoxicity

According to ISO-10993, the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay was performed to determine the *in vitro* cytotoxicity of the extracts of films. L929 cells were seeded into a 96-well plate at a density of 5000 cells per well and incubated with 100 μL culture medium containing either in the DMEM medium or the film extract fluid at 37 °C for various time periods. After the incubation, the culture medium in each well was removed and the cells were washed three times with PBS. 20 μL of MTT solution (5 mg/mL) was added to each well and cells were cultured for another 4 h. The supernatant was discarded and then 100 μL of DMSO was added to each well. The OD values of the plate were measured on an EIX-800 Microelisa reader at 570 nm (Bio-Tek Inc., USA).

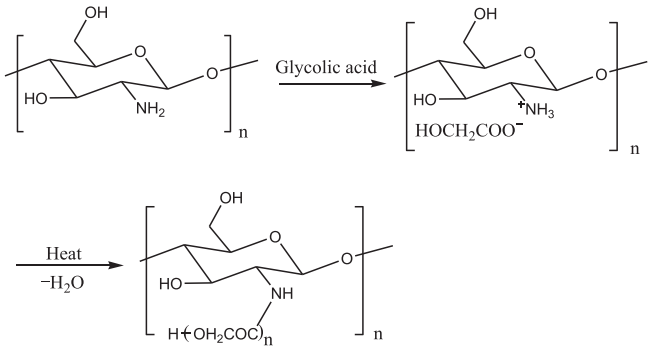
2.7. Bright-field microscopy measurement of cells

The cells were seeded onto the films, which had been placed into a 6-well plate at a density of 2.5×10^5 cells per well, and incubated for 12 h. Thereafter, the cells were observed by optical microscopy.

3. Results and discussion

The poly(glycolic acid) grafting chitosan were obtained after the reaction between chitosan and glycolic acid without using a catalyst (Scheme 1). When chitosan was dissolved in a glycolic acid aqueous solution, the amino groups of chitosan were protonated and the chitosan amino glycolic salt was formed [19]. The dehydration of the salt was done to form amide groups between chitosan and glycolic acid by heating the solution, and the polycondensation of glycolic acid was carried out simultaneously.

The different poly(glycolic acid) grafted chitosan films were prepared *via* the variation of the feeding molar ratios of glucosamine unit to glycolic acid (the molar ratios of glucosamine unit to glycolic acid was changed from 1/1 to 1/10). Fig. 1 shows the IR spectra of chitosan and the poly(glycolic acid) grafted chitosan copolymer films extracted with methanol. For the chitosan film (Fig. 1a), the absorption peaks at 1595 and 1658 cm^{-1} are attributed to the N–H bending vibrations of non-acylated NH_2 group and the carbonyl stretching of secondary amide (amide I band), respectively. Compared with chitosan film, the film of poly(glycolic acid) grafted chitosan (CS:GA = 1:1) (Fig. 1b) has new peaks appearing at 1639 and 1087 cm^{-1} corresponding to the carbonyl stretching of secondary amide (the NH_2 groups of chitosan unit are acylated by glycolic acid) and C–O stretching of glycolic acid (CH_2–OH), respectively. With the molar ratios of glucosamine unit to glycolic acid increased to 1/5 and 1/10 (Fig. 1c and d), the peaks located at 1749 and 1184 cm^{-1} can be assigned to the ester carbonyl stretching and C–O stretching of oligo(glycolic acid), indicating the formation of oligo(glycolic acid) attached to the chitosan. Additionally, no peak corresponding to ether groups from the reaction between the hydroxyl groups was found in the spectrum of the poly(glycolic acid) grafted



Scheme 1. Grafting copolymerization of chitosan and glycolic acid.