Carbohydrate Polymers 105 (2014) 63–69

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

In situ cross-linked polysaccharide hydrogel as extracellular matrix mimics for antibiotics delivery

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ARTICLE INFO

Article history:
Received 16 October 2013
Received in revised form
17 December 2013
Accepted 20 January 2014
Available online 29 January 2014

Keywords:
Hydrogel
Polysaccharide
Antibacterial
Wound dressing

ABSTRACT

Many synthetic hydrogels for drug delivery have been based on polyethylene glycol which is non-natural, non-biodegradable and only terminal-functionalizable. The polysaccharides dextran and chitosan not only are highly hydrophilic, biodegradable and pendant-functionalizable, but also more closely mimic the nature extracellular matrix glycosaminoglycans. Here, a biomimetic hydrogel based on chitosan and dextran was synthesized by the Michael addition reaction. The hydrogels have good swelling and cytocompatibility against NIH3T3. Moreover, vancomycin-loaded hydrogels were formed in situ, and could kill both Gram-positive bacteria and Gram-negative bacteria, indicating that the hydrogel as a wound dressing could provide protection against bacterial infection. Notably, the drug release was controlled via modifying the compositions. Therefore, the biomimetic polysaccharide hydrogels as a promising carrier have potential application for wound healing.

1. Introduction

The skin plays an important role in human body and prevents from being infected by microbes. Skin generally needs to be covered with a dressing immediately after it is damaged (Jayakumar, Prabaharan, Kumar, Nair, & Tamura, 2011). Wound healing typically occurs through the process of hemostasis, inflammation, tissue repair and remodeling. Conventional wound dressings such as bandages, gauze and foam dressing just cover the surface of the wound and absorb tissue exudates. However they cannot provide an appropriate environment for tissue repair and regeneration, easily adhere to wound and damage the new epithelial tissue leading to bleeding. Advanced dressings, including biological and synthetic scaffolds, can provide a physical barrier against secondary infection, as well as a compatible physiological environment (Song, Rane, & Christman, 2012). Wound infection is the major difficulty in the field of wound care management, because such infection can cause to form exudate, delay the wound healing, facilitate improper collagen deposition, etc. (Kumar et al., 2012; Madhumathi et al., 2010; Paul & Sharma, 2004). Hydrogels are soft and wet materials that contain a large amount of water for their three dimensional polymer network structure. Besides the applications such as drug delivery system, contact lenses, corneal implants, and as scaffolds for tissue engineering (Haque, Kurokawa, & Gong, 2012), they have been utilized as promising materials for wound healing, surgical tissue adhesives, and hemostasis during surgery as well as for local drug delivery depots (Ryu et al., 2011; Zhang, Qadeer, & Chen, 2011). Moreover, interpenetrated polymer networks have shown the promise as a way of incorporating antimicrobial agent into polymer such as the antibiotic streptomycin sulfate (Song et al., 2012) or tetracycline (Paul & Sharma, 2004) where the polymer networks act as a carrier for the antibiotics delivery system.

Chitosan is a modified natural polysaccharide derived from chitin and one of the rare natural-based cationic polymers (Schutz, Juillerat-Jeanneret, Kauper, & Wandrey, 2011). It has been widely used in biomedical field due to their bioactivity, biodegradability, biocompatibility, low toxicity and bacteriostatic effect (Bhattarai, Gunn, & Zhang, 2010; Brunel et al., 2009, 2010; Mao, Sun, & Kissel, 2010; Wu, Shen, Banerjee, & Zhou, 2010). The antibacterial activity of its derivatives against *E. coli* and *S. aureus* was affected by the degree of *N*-substitution and molecular weight, especially in the range between 5 and 10 kDa (Sajomsang, Gonil, & Tantayanon, 2009; Sajomsang, Tantayanon, Tangpasuthadol, & Daly, 2009; Tsao et al., 2010). Dextran is highly hydrophilic and has high molecular weight resulting in relatively good mechanical property (Rokhade, Patil, & Aminabhavi, 2007). Moreover, dextran is a natural biodegradable polysaccharide with abundant pendant hydroxyl groups amenable to chemical modification. More

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0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2014.01.068

importantly, the polysaccharide dextran is chemically similar to glycosaminoglycan which is an important constituent of extracellular matrix (ECM). We postulate that combination of chitosan and dextran in a composite hydrogel could more closely mimic the natural structure and function of ECM. In our previous study (Teng et al., 2010), the hydrogel based on thiolated chitosan and polyethylene glycol (PEG) was synthesized by the Michael type addition reaction, and the hydrogels have been used for drug delivery and cell tissue engineering.

Here, the hydrogels based on thiolated chitosan and maleic acid-grafted dextran were developed. The cell viability, swelling, drug encapsulation and release behaviors were tracked. Finally, the antibacterial property of drug-loaded hydrogels was further explored. We expect that the polysaccharide hydrogel as a wound dressing could promote wound healing.

2. Materials and methods

2.1. Materials

Chitosan (CS) was purchased from Golden-Shell Pharmaceutical Co. Ltd., Jiangsu, China. Dextran (Dex) was purchased from Sinopharm Chemical Reagent Co. Ltd., Beijing, China and dried under vacuum at 60 °C before used. 1-Hydroxybenzotriazole (HOBt) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC·HCl) were purchased from Medpep Co. Ltd., Shanghai, China. *N*-Acetyl-L-cysteine (NAc) was purchased from Alfa Aesar, USA. Maleic anhydride (Ma), triethylamine (TEA) and isopropyl alcohol (IPA) were purchased from Chemical Reagent Co. Ltd., Tianjin, China. 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich, USA. Dulbecco's modified eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS) and trypsin were purchased from Gibco, USA. Vancomycin was purchased from Aladdin, Shanghai, China.

2.2. Preparation of thiolated chitosan (CS-NAc)

N-Acetyl-L-cysteine conjugated chitosan (CS-NAc) was obtained according to the method reported by our group (Wang et al., 2009). Briefly, 500 mg of CS and 377 mg of HOBt were dispersed in water under stirring. Then 4286 mg of EDAC·HCl and 912 mg of NAc were added to the above solution. PH of the reaction mixture was adjusted to 5 using 1 M HCl, then reacting in the dark for 3 h. The resultant mixture was dialyzed (10 kDa cutoff) first against 5 mM HCl containing 2 μmol/L EDTA at 4 °C for 72 h in the dark, then 5 mM HCl containing 1% of NaCl, finally 1 mmol/L HCl before the solution was freeze dried. Thiol content was determined using Ellman's reagent as described by Krauland, Leitner, and Urch (2003). The main chemical shift of CS-NAc: Fig. S1 showed the ^{1}H NMR (400 MHz, D_2O, TMS): 4.94 (s, H-6), 3.99 (s, H-1, H-2), 3.83 (s, H-3, H-4), 3.24 (s, H-5), 2.97 (s, —S—CH_2—), 2.07 (s, —$COCH_3$). According to Fourier transform infrared (FTIR) result of CS-NAc, when compared with chitosan, a stronger absorption peak at 1654 cm^{-1} (amide I band) which was assigned to carbonyl in amide, indicating that NAc was introduced into chitosan successfully.

2.3. Preparation of maleic acid-grafted dextran (Dex-Ma)

Maleic acid-grafted dextran (Dex-Ma) was synthesized according to the method described by Kim, Won, and Chu (1999). Briefly, dextran was dissolved in 20 mL of DMF containing 2 g of LiCl at 90 °C under nitrogen. TEA was added to the solution as a catalyst at 60 °C and maleic anhydride was subsequently added. The reaction mixture was generally conducted at 60 °C for 10 h. The reaction mixture was precipitated in 50 mL of cold isopropyl alcohol, washed three times with isopropyl alcohol, and dried at room temperature under vacuum for a week and stored in cold dark before used. The degree of substitution of Dex-Ma was 55%, determined by acid-base titration. FTIR result of Dex-Ma shows that the peak at 1722 cm^{-1} was ascribed to carboxyl, and the peak at 1661 cm^{-1} was assigned to carbon–carbon double bound comparing with dextran. The ^{1}H NMR peaks at 6.3 and 6.6 ppm were protons of the double bond of maleic acid.

2.4. Preparation and characteristics of CNDM hydrogel

To determine the gelation time, the solution of CS-NAc and Dex-Ma (the molar ratio of thiol group to maleic group, 1:3, 1:2, 1:1, 2:1 and 3:1) in phosphate buffer solution (PBS, pH 7.4, 0.02 M) were incubated at 37 °C by vortexing. The gelation time was determined by a flow test utilizing a glass test tube inverting method reported by Jeong, Bae, and Kim (1999). As the solution lost fluidity in 1 min, the sample was regarded as a gel.

FTIR spectroscopy of the sample was performed with FTS 6000 spectrometer (Bio-Rad, USA). The dried samples were mixed with KBr and tableted. To examine thermal stability of hydrogels, the samples were measured using thermogravimetric analysis (TGA, Metzsh). Decomposition profiles of TGA were recorded with a heating rate of 10 °C/min in nitrogen between 20 and 450 °C.

2.5. Morphology of hydrogels

To observe the morphology of hydrogels, the synthetic hydrogels were quickly frozen in liquid nitrogen and further freeze-dried in a Freeze Drier (FTS SYSTEMS, USA) in vacuum at −90 °C for 36 h until all the solvent was sublimed. The freeze-dried hydrogels were then fractured carefully, and the interior morphology of the hydrogels were visualized by using a scanning electron microscope (SEM, Shimadzu SS-550, Japan) (Teng et al., 2010). Before the SEM observation, the hydrogel samples were fixed on conductive tape and coated with gold.

2.6. Swelling ratio of hydrogels

To understand the effect of the molecular transport of liquids into hydrogels on the drug release, swelling measurement was carried out by immersing the hydrogels in PBS (pH 7.4) at 37 °C. At predetermined time intervals, the samples were taken out and wiped carefully between tissue papers to remove the surface-adhered liquid droplets, and then weighted on an electron microbalance (AE 240, Mettler, Switzerland) to an accuracy of ±0.1 mg. Each sample was performed in triplicate, and average value was calculated for data analysis. The percentage of equilibrium water uptake was calculated as follows:

$$\text{Swelling ratio} = \frac{W_t - W_0}{W_0}$$

where W_t is the weight of swollen hydrogels, and W_0 is the initial weight of hydrogels.

2.7. The cell viability

Mouse fibroblast NIH3T3 cells were maintained in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 mg/L streptomycin, and 100 IU/mL penicillin. Cells were grown in a 25 mL cell culture flask and incubated at 37 °C in a humidified atmosphere of 5% CO_2 to approximately 70–80% confluence. After a subculture was performed every 2–3 days, medium