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A facile method to prepare polysaccharide-based in-situ formable hydrogels with antibacterial ability

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ABSTRACT

Polysaccharide-based in-situ formable composite hydrogels were prepared by a facile one-pot approach via Michael addition reaction, with maleilated chitosan and thiol derivatised hyaluronan. The hydrogels were characterized by Fourier transform infrared spectroscopy, Scanning electron microscopy, swelling ratio, oscillatory rheology and antibacterial activity. Results showed that the hydrogels with different molar ratios of free thiol/vinyl all formed in-situ within 15 min. With increasing of vinyl contents, gelation time and storage modulus increased, while porosity and swelling ratio decreased. Additionally, the hydrogels presented antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*, which will be benefit for biomedical applications.

1. Introduction

In-situ formable hydrogels have been employed as scaffolds, drug delivery systems, and cell carriers for wide biomedical applications [1,2] since they allow easy and homogenous distribution of bioactive molecules or cells within any defect size or shape and minimize the invasiveness of the surgical techniques [3].

In-situ formable hydrogels have been prepared by various chemical and physical crosslinking methods [4]. Photopolymerization of vinyl modified polymers is one of the most popular methods to achieve hydrogel formation at a defect site [5], irrespective of the potential cytotoxicity of photo-initiators and UV light [6,7]. Michael addition reaction is an increasing popular method to prepare hydrogels, wherein crosslinking is achieved by addition reactions between nucleophiles (e.g. thiol groups) and electrophiles (e.g. vinyl/acrylate groups) [8]. Poly(ethylene glycol) (PEG)-based hydrogel systems have been mostly studied, but the multistep of hyper-branched macromers synthesis, functionalization and purification [9,10], undoubtedly increases the cost and complexity of preparation process.

Naturally derived polymers have showed great advantages over common synthetic polymers [4,11], such as better biocompatibility, tailored enzymatic biodegradation, etc. It is worthy to be noted that, comparing with PEG, the chemically reactive multi-groups on such water solvable macromolecules offer more crosslinking points to form large network. Therefore, the design of natural polymers with desired functional groups to provide crosslinking in situ is highly advantageous, which can create a more biomimetic microenvironment for biomedical applications. Additionally, hydrogels with inherent antibacterial activity are even more beneficial since they can absorb biological fluids, avoid water loss and microbial invasions, and offer adequate gaseous exchange when exposed to the environment [12]. Herein, we propose a novel, facile one-pot approach to prepare in-situ forming antibacterial hydrogel via self-crosslinking of functionalized polysaccharide derivatives, without using any extraneous chemical crosslinking agents.

2. Experimental section

Materials, specific syntheses and characterization of maleilated chitosan (CS-MA) and thiol derivatised hyaluronan (HA-SH) are provided in the Supporting information. Chitosan (CS) was modified with vinyl carboxylic acid groups [13], while hyaluronan (HA) was functionalized with thiol groups [9,14]. The functionalized polysaccharide derivatives were characterized by ^{1}H nuclear magnetic resonance (^{1}H NMR), Fourier transform infrared spectroscopy (FT-IR), Zeta potential and UV–visible spectrophotometric measurements.

2.1. Preparation of polysaccharide hydrogels via Michael-type thiol-ene reaction

The concentration is defined as the total dry weight of both HA-SH and CS-MA per volume of deionized water. CS-MA and HA-SH

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were separately dissolved in deionized water, and then were mixed in vials by vortexing for 1 min at a final polymer concentration of 20 mg/mL corresponding to the molar ratio of free thiol/vinyl of 4:1, 4:2, 4:3, and 4:4, respectively. Afterwards, 58% (wt%) beta-glycerophosphate disodium salt (β-GP) aqueous solution was added to adjust the pH to 7 and the mixtures were incubated at 37 °C for 24 h. The time for gel formation (denoted as gelation time) was determined using the vial inverting method [8]. Rheological experiments were performed with a Kinexus Pro rheometer (Malvern, UK) using parallel plates (Ø 20 mm). The time-sweep of precursor solution was carried out at 37 °C, a frequency of 1 Hz, and a strain of 1%. The modulus values versus frequency analyses of hydrogels were carried out at 37 °C, a strain of 1%. Scanning electron microscopy (SEM, PHILIPS XL-30ESEM, the Netherlands) was employed to determine the morphology of freeze-dried hydrogels, which were kept at −80 °C for 24 h prior to be freeze-dried. Swelling ratio (Q) of the hydrogel was determined as described in literature [9], which was defined as $(W_s/W_d)\times 100\%$ (the weight of swollen hydrogel/the dried weight of hydrogel). The antibacterial activities of hydrogels against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) were measured as described in literature (details in Supporting information) [15], which was calculated by the equation: killing percentage $(\%)=[(N_{control}-N_{sample})/N_{control}]\times 100\%$ ($N_{control}$ and N_{sample} are the number of colony forming units at the end of the incubation period on the Luria-Bertani agar plates of the control and hydrogel samples, respectively).

3. Results and discussion

3.1. Characterization of CS-MA

The chemical composition of CS-MA was characterized by ^{1}H NMR and FT-IR. Compared with the spectrum of pure CS, a new peak of –CH=CH– at δ=6.3 [16] was observed, as Fig. 1(a) shows. The determined substitution degree of CS-MA from ^{1}H NMR was 65%, by comparing the integrals of signals at δ=3.0 (C2 proton of

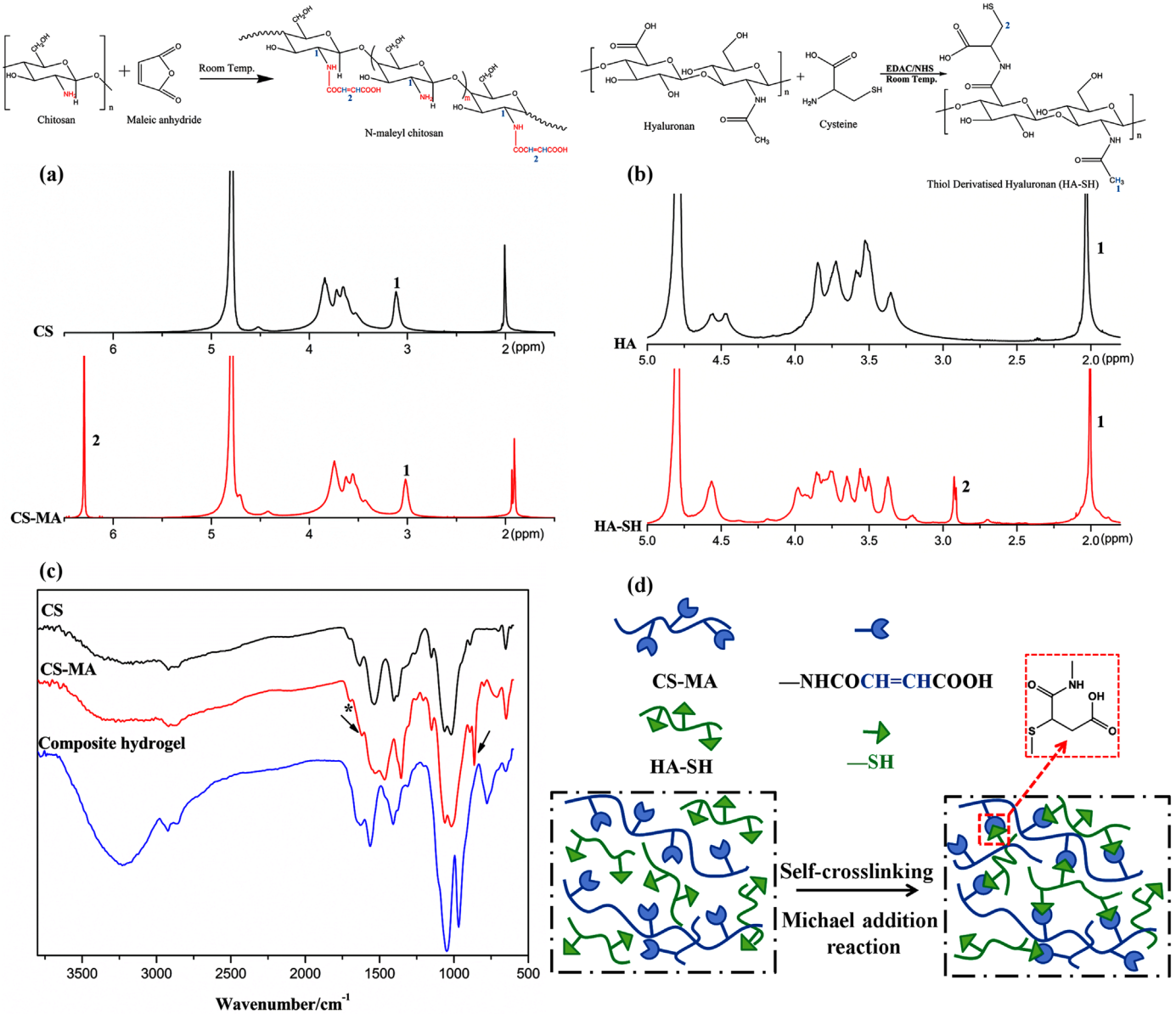

Fig. 1. Synthesis schemes and ^{1}H NMR spectra of CS-MA (a) and HA-SH (b), FT-IR spectra (c) of CS, CS-MA and composite hydrogel ($n_{free\ thiol}/n_{vinyl}$=4:4), and synthesis scheme of the hydrogels (d).

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