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Functional hyaluronic acid hydrogels prepared by a novel method

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ABSTRACT

In this study, a novel simple method was developed to prepare functional hyaluronic acid (HA) hydrogels simultaneously containing hydrazone and disulfide bonds in their crossbridges. The HA hydrogels were formed by directly reacting 2,5-hexanedione and 3,3′-dithiodipropionate hydrazide-modified HA, and were characterized by FT–IR, SEM, TGA and mechanical tests. The results showed that the formation of HA hydrogels was a result of the reaction between ketone and hydrazide groups. The resultant HA hydrogels exhibited a porous morphology with a pore size range of 50 μm to 400 μm, and their compressive modulus and G″/G′ ratio were 18.8 ± 0.6 kPa and 0.002, respectively. Both swelling and degradation ratios gradually decreased with the increasing degree of crosslinking. However, the degree of crosslinking had a slight effect on the decomposition temperature of the HA hydrogels. It can be concluded that the simple method presented in this study is feasible to prepare HA hydrogels through hydrazone bond crosslinking by reacting diketone molecules and hydrazide-modified HA, and the HA hydrogels have potential in biomedical applications.

1. Introduction

Being water-insoluble polymer networks, polymer hydrogels arise from hydrophilic polymer or macromonomer by crosslinking and contain more than 90% water just like living tissues [1–3]. Because of the native biocompatibility and diverse structure design of hyaluronic acid (HA), HA hydrogels have been extensively applied in biomedical fields, such as osteoarthritis treatment [4], ophthalmic surgery [5,6], skin filling [7], drug/protein delivery [8–12] and tissue regeneration [13]. Currently, the crosslinking methods for preparing HA hydrogels can be divided into two categories: physical crosslinking and chemical crosslinking. The formation mechanisms of HA hydrogels produced by physical crosslinking methods mainly include hydrogen bonds [14] and hydrophobic interactions [15]. However, the mechanical strength of these HA hydrogels is poor. Therefore, many chemical crosslinking methods were developed to improve the mechanical properties of HA hydrogels, such as photochemical crosslinking, carbodiimide crosslinking, azide–alkyne cycloaddition and thiol-ene addition reactions [16–20]. A major concern is still that the residual photoinitiator, catalyst, activating agent or their decomposition product during crosslinking would have deleterious consequences for the biological activity of HA hydrogels [21]. Recently, it was reported that a gentle crosslinking method, namely hydrazone bond crosslinking, could produce HA hydrogels [22]. The HA hydrogels were formed just by mixing the solutions of HA aldehyde and HA adipic dihydrazide without additional chemical agents, and had great potential in biomedical fields due to their non-cytotoxicity. Importantly, a recent study indicated that the hydrogels formed from ketone and hydrazide had better biocompatibility than those formed from aldehyde and hydrazide [23]. To the best of our knowledge, preparing HA hydrogels containing both hydrazone and disulfide bonds in crossbridges by reacting diketone and hydrazide-modified HA has not been reported.

Herein, we reported a novel simple method to prepare HA hydrogel by reacting 2,5-hexanedione and 3,3′-dithiodipropionate hydrazide-modified HA (DTPH-HA) in the absence of any chemical catalyst. The HA hydrogels not only possess both hydrazone and disulfide bonds in their crossbridges, but also bear hydrazide and disulfide bonds in residual pendant side groups which may be used for further biodecoration. The HA hydrogels with different degrees of crosslinking were prepared and characterized by Fourier transform infrared spectroscopy (FT–IR), scanning electron microscope (SEM), thermogravimetric analyzer (TGA), mechanical tests, swelling and enzymatic degradation assays.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium (HA, molecular weight: 300 kDa), 2,5-hexanedione (>99%) and N-hydroxysuccinimide (NHS) were purchased from Aladdin Reagent Inc. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) was purchased from Sigma-Aldrich. 3,3′-dithiodipropionate hydrazide (DTPH) was synthesized according to our previously reported procedure [24]. All other chemicals were of analytical grade and were used

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without further purification. All aqueous solutions were prepared using ultrapure water with a resistance of 18.25 MΩ.

2.2. Synthesis of HA hydrogels

HA hydrogels were prepared via hydrazone bond crosslinking by reacting ketone groups of 2,5-hexanedione and hydrazide groups of DTPH-HA. DTPH-HA was first synthesized according to our previously reported procedure [24]. Briefly, HA (400 mg, 1 mmol repeating units), DTPH (1.43 g, 6 mmol) and N-hydroxysuccinimide (69 mg, 0.6 mmol) were dissolved in 40 ml deionized water, and the pH of the solution was adjusted to 4.5–5 by adding 1 mol/L HCl. After that, EDC·HCl (115 mg, 0.6 mmol) was added, and the mixture was stirred for 24 h at room temperature. The product was obtained after dialysis against distilled water for 72 h followed by lyophilization. The substitution degree calculated from the ^{1}H NMR spectrum was about 40%.

HA hydrogels were prepared as follows: DTPH-HA (60 mg, 51.6 μmol hydrazide groups) was dissolved in deionized water (2 ml), and then the crosslinking agent 2,5-hexanedione (6.5, 13 or 19.5 μmol) was added. The mixtures were vigorously vortexed and subsequently defoamed, and light yellow hydrogels were formed within 60 s.

2.3. Characterization of HA hydrogels

2.3.1. FT–IR analysis

FT–IR spectra were recorded on a Shimadzu IR Prestige-21 FT–IR spectrometer in transmittance mode in the frequency range of 1200–1800 cm^{-1} with 2 cm^{-1} resolution. The measurements were done on dried samples in analytical grade KBr pellets by accumulation of 32 scans.

2.3.2. Morphology

The morphology of freeze-dried HA hydrogel samples was observed by scanning electron microscopy (SEM, S-3400 N, Hitachi, Japan) at an accelerating voltage of 5 kV.

2.3.3. Thermostability

Thermogravimetric analysis (TGA) was performed from room temperature to 500 °C by using Mettler Toledo TGA1 equipment at a heating rate of 10 °C/min in nitrogen atmosphere. Samples were accurately weighed (5–10 mg) in crucibles.

2.3.4. Compressive behavior

The compressive behavior was measured using Instron 5943 universal testing instrument at a constant rate of 1 mm/min. The slopes of the stress–strain curves from 10% to 20% deformation were used to calculate the compressive modulus values.

2.3.5. Viscoelasticity

Rheological characterization was performed using a TA instrument DHR-2 rheometer in a frequency range of 0.1–10 Hz. The storage modulus (G′) and loss modulus (G″) were recorded and used to calculate G″/G′ ratios.

2.3.6. Equilibrium swelling

Swelling test was carried out in PBS (pH = 7.4) solution at 37 °C. Five samples as a group were measured to obtain an average value. The hydrogel samples were taken out at predetermined time points, and weighted after surface water was removed with filter paper. The swelling ratio was calculated by using the following equation:

$$\text{Swelling ratio}\ (\%) = [(W_1 - W_0)/W_0] \times 100$$

where, W_0 and W_1 were dried weight and swollen weight of HA hydrogels, respectively.

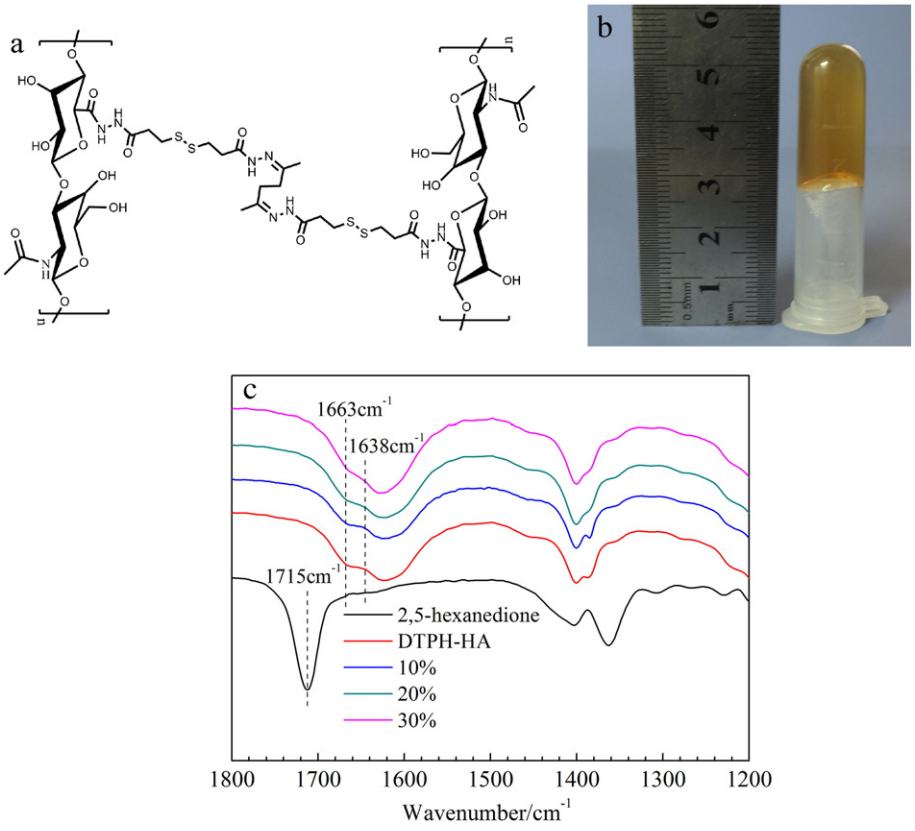

Fig. 1. (a) Chemical structure and (b) photograph of HA hydrogel; (c) FT–IR spectra of 2,5-hexanedione, DTPH-HA and HA hydrogels with different degrees of crosslinking.