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RESEARCH ARTICLE

Fabrication and characterization of hyaluronic-acid-based antigen sensitive degradable hydrogel

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Abstract Biomolecule sensitive hydrogel, as an important part of intelligent materials, has extensive applications in the biochemical and biomedical fields. In this paper, an antigen sensitive degradable hydrogel based on hyaluronic acid (HA) has been prepared. Antigen and antibody were covalently attached to the hydrogel simultaneous, while HA was cross-linked used adipic dihydrazide (ADH) as cross-linking agent primarily. We describe the synthesis, characteristics, and the antigen sensitive behavior of the hydrogel. The hydrogel can exhibit a reversible swelling behavior while the native antigen is present or not. This property of the antigen sensitive hydrogel may be applied as novel drug delivery system, which can release drug by the presence of native antigen.

Keywords intelligent hydrogel, hyaluronic acid, antigen sensitive

1 Introduction

The stimuli-sensitive hydrogels can respond to the pH [1–5], temperature [6–9], electric field [10,11], glucose [12], antigen [13,14], and so on. This property has many applications, such as an intelligent drug release system. Antigen sensitive hydrogels have a similar property that exhibits remarkable volume changes. There have been two types of the antigen sensitive hydrogel of undegradable hydrogel. One is antigen-antibody entrapment hydrogel of acrylamide (AAm) and N, N'-methylenebis (acrylamide) (MBAA) [14], and the other is antigen-antibody semi-interpenetrating polymer network (semi-IPN) hydrogel of AAm and MBAA [13]. The former does not show the reversible volume change, while the latter does. It is still worthy to investigate the properties of antigen-sensitive hydrogels by changing the polymeric materials and approach to preparing hydrogels. The idea to make degradable antigen sensitive hydrogel is very important in tissue engineering, regenerative medicine technology, and new application of biomaterials. In the present paper, we report an antigen-sensitive degradable hydrogel in the system of HA immobilized with IgGs.

We used the only carbohydrate moieties at each heavy chain of IgGs to immobilize to HA hydrogel, which is a covalent site-directed attachment. The region of oxidated carbohydrate moieties is far away from the combining sites (F_{ab}) of IgGs. Therefore, the antibody and the antigen can keep their combining sites toward the mobile phase and keep their bioactivities well. Substrate of HA is selected as an example to study that its well biocompatible and biodegradable.

Figure 1 is the strategy of synthesizing the hydrogel. The reversible binding between antigen and antibody is used as the cross-link mechanism in an IPN hydrogel. HA was primarily cross-linked using ADH that is cross-linking agent. Then, it was further cross-linked by the reciprocity between antigen and antibody. We used rabbit IgG and goat-anti-rabbit IgG as the antigen and antibody. The hydrogel was soaked in the solution of antigen and antibody oxidized to immobilize. The binding of the IgGs formed the stimuli-responsive cross-link point in the hydrogel.

Our typical method to prepare antigen-antibody IPN hydrogel is shown in Fig. 2. The hydrogel can be swelled in the presence of the free antigen in media because the intrachain antigen-antibody binding can be dissociated by the exchange of the grafted antigen for free antigen, so the flexibility of the polymer chains in the hydrogel leads to the change of the polymer chains in the hydrogel. On the other hand, the osmotic pressure was changed by the antigen both free and grafted, which makes the hydrogel shrink. These two factors make the opposite results and

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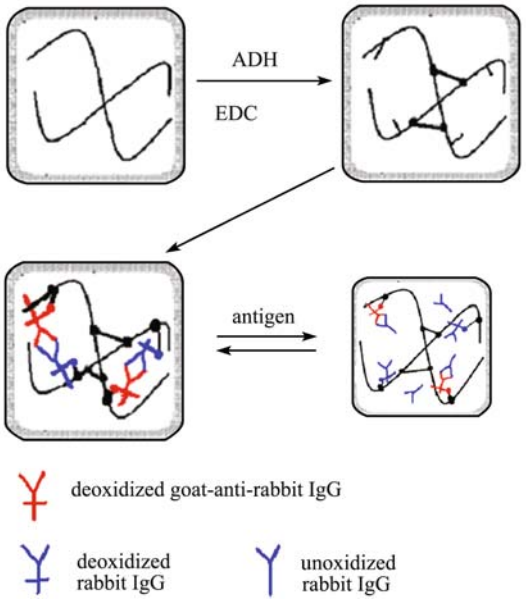

Fig. 1 The strategy in synthesizing the hydrogel: Add hydrazide cross-linker (e.g., ADH) to HA solution. Then adjust pH to 3.5–4.75 by adding 0.1 mol/L HCl. Add EDC to the mixture. Then, the polymer hydrogel was lyophilized. The hydrogel was soaked in the solution of oxidized antibodies in PBS and left overnight under rotary mixing at 4°C–8°C. Afterward, the hydrogel was washed three times with 0.1 mol/L PBS.

achieve the balance last. In the absence of a free antigen, the hydrogel will make a reversible behavior that the point will be associated again.

2 Materials and methods

2.1 Materials

HA sodium salt (MW 2.6–2.7 million Da) was purchased from SHANDONG FREDA BIOCHEM. The ethyl N, N-dimethylaminopropyl carbodiimide (EDC), Sodium borohydride, and ADH were obtained from Sigma (St. Louis, MO).

2.2 Preparation of the HA-ADH hydrogel

The method has been reported previously [15]. HA was dissolved in water, and then hydrazide cross-linker was added (e.g., ADH). After adjusting the pH value to 3.5–4.75 by the addition of 0.1 mol/L HCl, the carbodiimide reagent (EDC) was added to the mixture. Using EDC as an activating agent at pH = 4.75, ADH was coupled with HA, formed the HA-ADH, and HA-ADH-HA [16]. After stirring thoroughly, the mixture was allowed to gel at room temperature. The final polymer was washed with deionized water for five times and then immersed in deionized water overnight to remove the residue. Then, the polymer hydrogel was lyophilized.

2.3 Preparation of oxidized antibody

The antibody containing aldehyde groups was prepared by oxidation of saccharide moieties in the Fc part of the antibody by the reaction with sodium periodate [17]. A 10 mg of antibody was dissolved in 1 mL sodium acetate buffer (0.02 mol/L acetate buffer with 0.15 mol/L NaCl, pH 5). NaIO4 was added to reach the final concentration 0.02 mol/L. The solution was stirred in the dark at 2°C–8°C for 2 h. Then, 25 mL ethylene glycol was added to remove excess sodium periodate.

2.4 Immobilization of antibodies

The first process of immobilized antibodies has been reported previously [18]. Hydrogel was soaked in the solution of oxidized antibodies in 5ml volume of PBS (pH 7.4, containing 1 mol/L NaCl) and left overnight under rotary mixing at 4°C–8°C. Afterward, the hydrogel was washed three times with 0.1 mol/L PBS. After the antigens had been immobilized on the hydrogel, the hydrogel was dipped into the sodium borohydride solution (0.5 mg/mL) to deoxidize the bond.

2.5 Infrared (IR) spectroscopic measurement

Fourier-transformed IR spectra of untreated or treated HA membranes were measured to confirm the expected pendant hydrazide amino functionalities arrayed along the hyaluronate back-bone amide bond formation in the cross-linked hydrogel. IR spectra were obtained with BIO RAD (FTS 135, Cambridge, MA, USA).

2.6 Microstructure of hydrogel

We used the SEM (JSM-6460LV) to scan the sample of HA before and after immobilizing the antigen and antibody.

The porosity was evaluated by the absorption of the water and mercury intrusion. A block of weighed dry gel (W_{dry}) was immerged into PBS (pH = 7.2). After balancing for three hours, the hydrated gel was bolted off the free water on the surface and weighted (W_{wet}). The hydrated ratio was calculated by the formula: $H=(W_{wet}-W_{dry})/W_{wet}$.

2.7 Pore distribution of hydrogel

The distribution of the pores in the hydrogels in dry state was measured by mercury injection apparatus (AutoPore IV, Micromerities Instrument Co., USA). There are two samples. One is HA-ADH without antigens, and the other

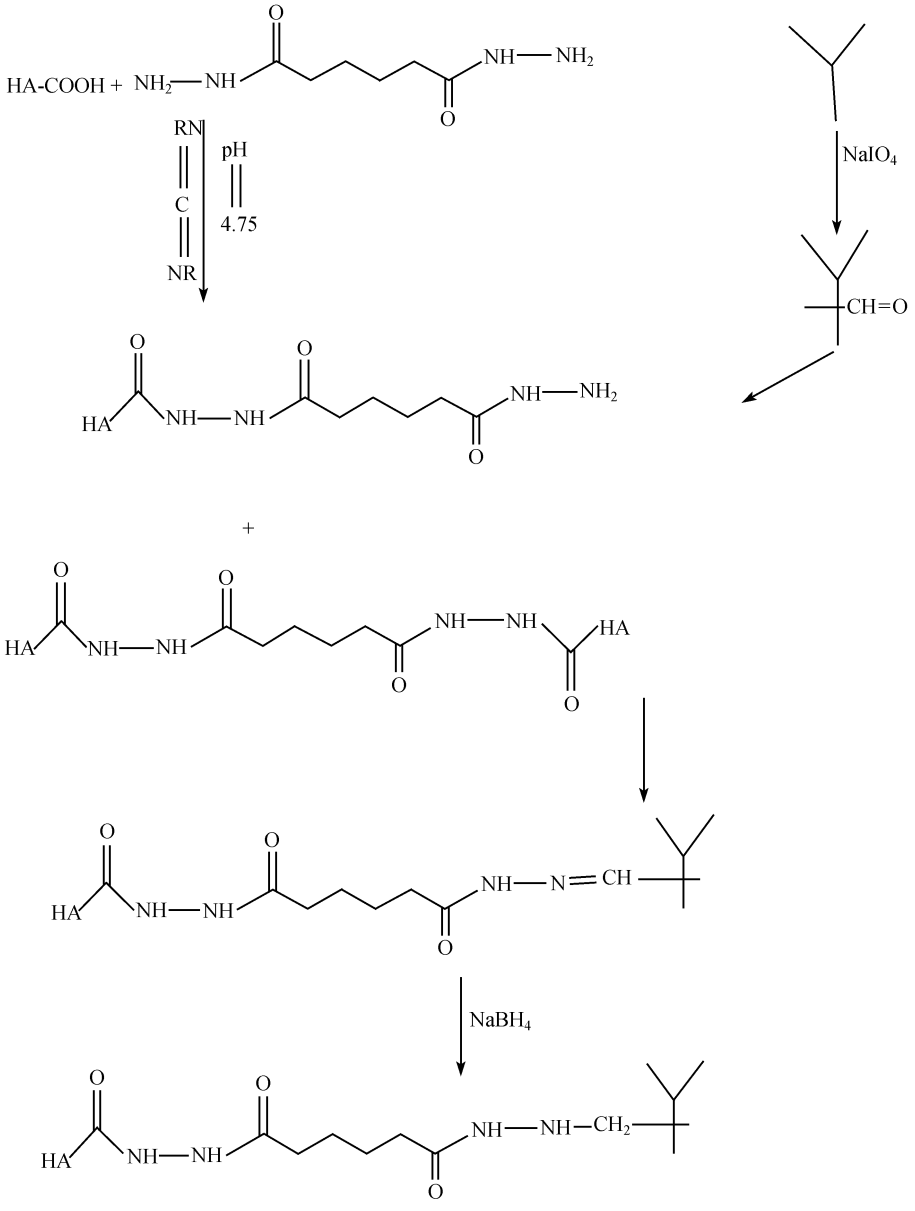

Fig. 2 The typical method to prepare the hydrogel

is HA-ADH immobilized with antigens. V% is defined as the fraction of volume of pore with specific diameter.

2.8 Swelling measurements

The samples were immersed in vials (100 mL) controlled with solutions. The vials were set in a temperature-controlled bath at 37°C for three hours to reach the equilibrium swelling. For the samples immobilizing without and with antigens, the swelling rate was measured using the method as described in section 2.6 ($n = 6$). In addition, the effect of rabbit IgG concentration was evaluated ranging 0.1–0.5 mg/mL.

To test the reversibility of the hydrogel, 12 samples were immersed in pure PBS and rabbit IgG (0.5 mg/mL) in turn for three hours to reach the equilibrium and measured these different swelling rates used the method, as described in Section 2.6.

3 Results

3.1 Hydrogel characterization

The prepared HA-based antigen sensitive hydrogel was scanned by SEM. Figure 3 shows that both hydrogels were

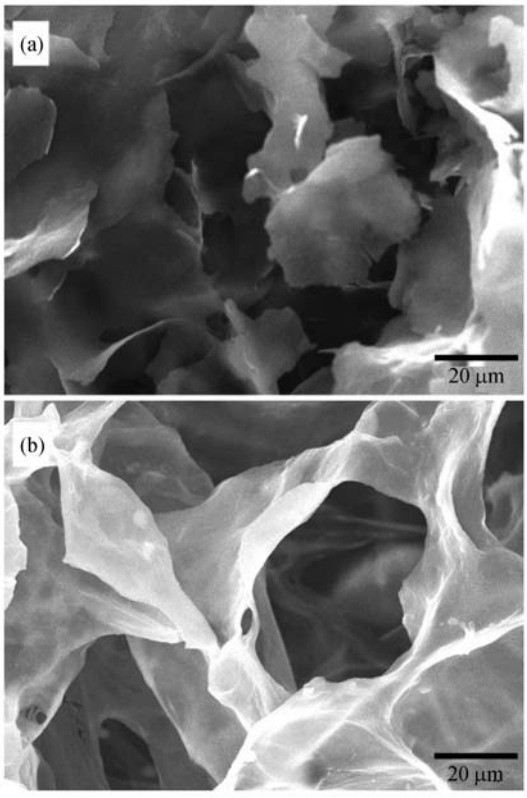

Fig. 3 SEM imagines of HA and HA-ADH hydrogel: **(a)** HA hydrogel; **(b)** HA-ADH hydrogel

porous. Both kinds of the hydrogels have a pore diameter of about 100 μm, and little pore has diameter less than 10 μm (shown in Fig. 4). The hydrogels featured flat surface before immobilization. While after immobilization, the surface of the hydrogel changed to crimped, and the pore distribution of the hydrogels changed. More pores have a diameter of 50 μm, while lesser pores have a diameter of 200 μm (shown in Fig. 4).

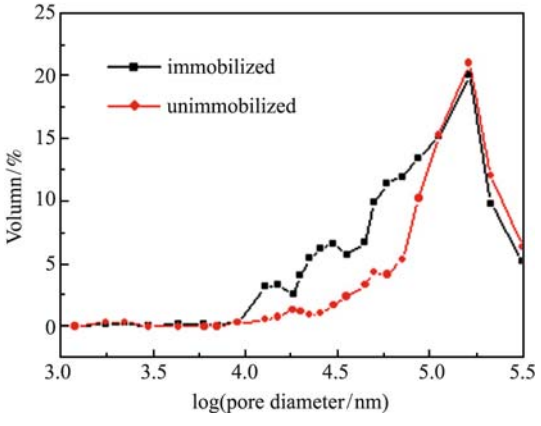

Fig. 4 The distribution of pores in the dry-state hydrogels measured by mercury injection apparatus

3.2 Infrared result

FTIR spectroscopic studies were conducted to monitor the chemical modifications in hydrogel structure at molecular level due to cross-link. It is indicated that the hydrogel cross-linked have two new vales at 3310 and 3228 cm^{-1}, as shown in Fig. 5. After ADH has cross-linked to HA, the amidocyanogen shifted from 3310 to 3228 cm^{-1}.

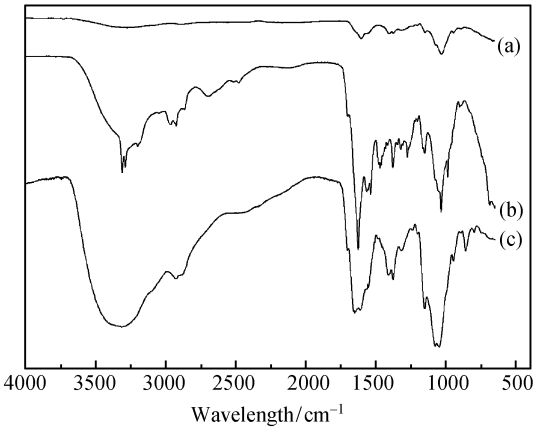

Fig. 5 Infrared spectra of **(a)** HA, **(b)** HA cross-linked with ADH, and **(c)** HA-ADH immobilized by antibodies

3.3 Swelling result

The material was further cross-linked for the immobilization, leading to the swelling rate increase from 75.0%± 4.5% to 88.1%±2.0% (Fig. 6). When the native antigen is present, the swelling rate decreased (Fig. 6) at 3 rabbit IgG concentrations.

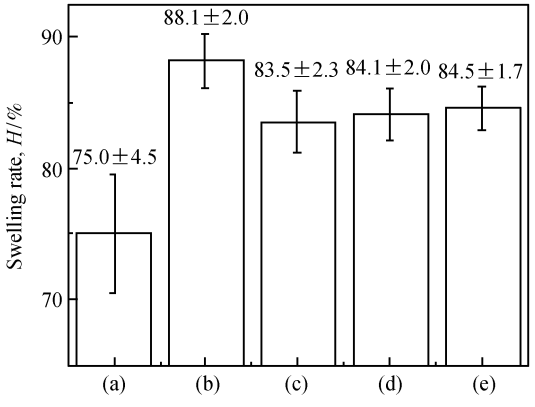

Fig. 6 The swelling rate of different samples in variational concentrations of antigen ($n = 6$): **(a)** hydrogel without antigen; **(b)(c)(d)(e)** hydrogels with antigen (The solutions of (a) and (b) are PBS, while those of (c), (d), and (e) are antigen solutions with the concentration of 0.1, 0.2, and 0.5 mg/mL, respectively.)

Figure 7 shows that the swelling rate change of the hydrogel was reversible. When the antigen is present, the swelling rate increased. After the antigen disappeared, the swelling rate returned back to original swelling rate.

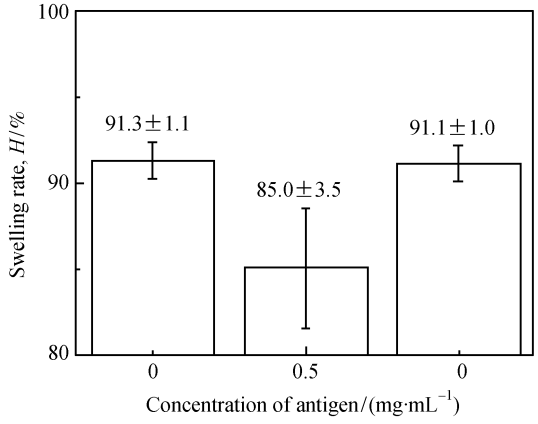

Fig. 7 The swelling rate of HA-based antigen sensitive hydrogels in variational concentrations (0 and 0.5 mg/mL) of antigen (n = 12)

4 Discussion

The hydrogel conjugated with antibody was successfully prepared by the reaction of hydrazide groups introduced to hydrogel (Fig. 2). ADH can link to the molecule bone of HA at a mild condition, remaining some dissociative groups. Figure 5 indicates that some amidocyanogen cross-linked but some remained. The amidocyanogen that remained was used to immobilize the antigen and antibody later. The antibodies were pretreated with oxidation of saccharide units of the glycoprotein at the F_c fragment. After immobilization, the cross-link bond was deoxidized to stabilize the immobilization. Therefore , the antigen and antibody will not break off from hydrogel easily. At the same time, the antigen and antibody cross-linked. Figure 4 shows the result that the pore diameter was reduced. The diameter-reduced pore was the result of the cross-link between antibody and antigen. In a word, this hydrogel can be synthesized at a mild condition. It keeps the activity of antigen. It is easy to adsorb some biomolecules without reduce the activity of the molecules.

A prominent transition of the hydrogel structure in response to antibody is demonstrated by the reversible swelling rate change. When the native antibody was present, the swelling rate of the hydrogel decreased (shown in Fig. 7). With the presence of native antigen, it competed with the antigen immobilized. Some of the original bonds were dissociated, while some of free native antigens were associated in the hydrogel. The free native antigen bond with antibody immobilized and the disconnection between immobilized antigen and antibody change the osmotic pressure as well as the flexibility of the polymer chains in the hydrogel, resulting to the swelling rate change that show the reversible swelling behavior. In this hydrogel, the shrinking behavior is more evident than the flexibility of the polymer chains. However, if the crosslink rate of hydrogel decreases, the flexibility of HA chains may be more efficient than osmotic pressure. In other words, we can change the crosslink rate to synthesize hydrogel which will shrink or not in the presence of the antigen. This property of the hydrogel may be useful in some applications. Further studies on the mechanism of the interaction and balance of osmotic pressure and the flexibility of the chains will be focused on.

The HA antigen sensitive degradable hydrogel is a model that shows the advantages. First, HA, as the substrate of hydrogel, has been demonstrated to well biocompatibility. Other biomaterials can also be used as the substrate. Second, the preparation procedure is simple and rapid at mild condition, and the immobilization is covalent. Many biomolecules are easy to lose their activities. Thus, a mild condition during synthesis is important for further applications to protect the activity. Finally, the swelling behavior responded to native antigen is reversible. The hydrogel keep sensitive to native antigen for a controllable time before degradation, because the degradation rate can be adjusted by the first step of cross-link [19].

5 Conclusions

In this paper, we describe a novel model of an antigen sensitive hydrogel. The method of immobilizing the antigen retains the biological activity of antigen for its mild reaction condition. Except for the same sensibility with other antigen sensitive hydrogel, this hydrogel can degradate *in vivo* as a biomaterial. Using this model, a novel drug release system would be developed, and the antigen sensitive hydrogel is respected to have potential applications in future.

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