Aldehyde-Sodium Alginate and Amino-Gelatin Preparation as Soft Tissue Adhesive

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Abstract: Sodium alginate and gelatin are both remarkable natural biomaterials; they all have been extensively applied in tissue engineering and other relative fields, due to their low price and good biocompatibility. In this paper, we oxidized sodium alginate with sodium periodate to convert 1, 2-hydroxyl groups into aldehyde groups to get aldehyde-sodium alginate (A-SA). Gelatin was modified with ethylenediamine (ED) in the presence of water-soluble 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC) to introduce additional amino groups to get amino-gelatin. Upon mixing the A-SA and amino-gelatin aqueous solutions together, a gel rapidly formed based on the Schiff's base reaction between the aldehyde groups in A-SA and the amino groups in amino-gelatin. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the characteristic peak of Schiff's base group in the hydrogel. The gelation time measure has confirmed the gelation time is dependent on the aldehyde group content in A-SA and amino group content in amino-gelatin. The fasted hydrogel formation takes place within 30 s. The entire test suggested that this gel could be a promising candidate as soft tissue adhesive.

Key words: *oxidized sodium alginate*; *amino-gelatin*; *adhesive agent*

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Introduction

Each year more than 12 million traumatic wounds are treated. These injuries have traditionally been closed with sutures[1]. In the past few years, many researches[2-7] have been carried out on polymeric adhesives which can be used for the closure and protection of wounds. Some of these have been tried in hard tissue. Generally speaking, these adhesives have usually proven to be too toxic, too weak, or too short-lived to function effectively as soft adhesive[8]. So it is necessary to prepare a kind of polymeric adhesives for wounds closed as soft tissue adhesive.

Hydrogels are a kind of materials that have gained widespread application as tissue adhesives due to their highwater content and physical properties similar to native extracellular matrix (ECM)[9-10]. Fluid balance in injury is very important since heavy loss of water from the body by exudation and evaporation may lead to a decline in body temperature and an increase in the metabolic rate[11]. Besides this, soft tissue adhesive should have certain other properties like ease of application and removal, and proper adherence so that there will not be any area of non-adherence left to create flxuid-filled pockets for the proliferation of bacteria[12]. A soft tissue adhesive can effectively stop bleeding by a simple procedure[13]. It is a dynamic process and the performance requirements of a dressing can change as healing progresses. However, it is widely accepted that a warm, moist environment encourages rapid healing and most modern wound care products are designed to provide these conditions[14-15].

Alginate are anionic linear polysaccharides with 1, 4-linked β-D-mannuronate (M) and 1, 4-linked α-L-guluronate (G) residues in varying proportions. Gelatin has many amino and carboxyl groups which provide modified site. In this paper, the gel was prepared by aldehyde-sodium alginate (A-SA) and amino-gelatin. The gelation time of soft tissue adhesive was changeable with the A-SA and amino-gelatin. Sodium alginate was partially oxidized by sodium periodate. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the characteristic peak of aldehyde group in the ASA. Additionally, the oxidized degree (OD) or the aldehyde contents were measured by using titration methods. The aldehyde content of ASA increased with the increasing amounts of sodium periodate added. A-SA had a controllable biodegradability under physiological condition. The amino contents of amino-gelatin were measured by using trinitro-benzene-sulfonic acid (TNBS) method. Amino contents increased with the increasing amounts of ethylenediamine (ED). The introduction of amino groups in gelatin improved its solubility and facilitated gelatin dissolving in water at room temperature. Gelation occurs by Schiff's base reaction between aldehyde groups of A-SA and amino groups of amino-gelatin to form self cross-linking hydrogels as soft tissue adhesive. These can promptly form a gel and firmly bond to soft tissue when mixed. The gelation time is dependent on the aldehyde group content in A-SA and amino group content in amino-gelatin. In this method, wound dressings that can be formed *in situ* offer several advantages over the use of preformed dressings such as conformability without wrinkling or fluting in the wound bed, ease of application, and improved patient compliance and comfort.

1 Experimental

1.1 Materials

Sodium alginate (medium viscosity grade), gelatin (from pigskin), sodium periodate, ED and 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC) were obtained from Sigma. All other chemicals were reagent grade and used as received.

1.2 Preparation of A-SA

A-SA was prepared according to the method of Biji Balakrishnan groups used previously[12-13]. Estimation of OD or aldehyde content was done by iodometric titration and hydroxylamine hydrochloride method[14]. In brief, different mass of $NaIO_4$ dissolved in 100 mL of water was added dropwise to 100 mL of 5 g sodium alginate solution (molar ratio of sodium alginate and $NaIO_4$ was respectively 4∶2, 4∶3, 4∶4, 4∶5, and 4∶8); the solution was then stirred for 6 h at room temperature and shielded from light. After that, 2 mL of

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ethylene glycol was added to terminate the reaction. Lastly, the A-SA solution was dialyzed (cut off $M_w \approx 14\ 000$) for 3 d against water, lyophilized to obtain the final product (yield: 60%). The OD of A-SA was determined by the method mentioned above.

1.3 Preparation of amino-gelatin

Amino-gelatin was prepared according to our previously described [14]. Briefly, 5 g of gelatin was dissolved in 100 mL (0.1 mol/L) sodium dihydrogen phosphate (NaH_2PO_4) solutions (pH = 5). Different amounts of ED and EDC were added, and then the pH was adjusted back to 5 by hydrochloric acid. The molar ratio of carboxyl groups (COOH) on gelatin chains, EDC, and ED was respectively 1∶2∶1, 1∶2∶5, 1∶2∶20, and 1∶2∶100. The reaction was allowed to proceed at room temperature overnight. Hereafter, the result mixture was dialyzed (cut off $M_w \approx 3\ 500$) against ultrapure water for 3 d to remove the excess ED and EDC, the mixture was then freeze at −80 ℃, lyophilized and stored. The amino content in the amino-gelatin was determined by TNBS method according to our previously report [14].

1.4 Gelation time measurement

The weight contentration 40% of A-SA solution was prepared by dissolve 4 g oxidized sodium alginate in 10 mL water at room temperature. The weight contentration 50% of amino-gelatin solution was prepared by dissolve 5 g amino-gelatin in 10 mL water at room temperature. After homogenizing, the mixture were poured into molds and finally incubated at 37 ℃ to determine the gelation time.

2 Results and Discussion

2.1 The modified and characterization of A-SA

The schematic of preparation A-SA was shown in Fig. 1. The reaction schemes suggested that sodium alginate required one periodate molecule to open one monomer unit (or repeating unit) of sodium alginate to form two aldehyde groups.

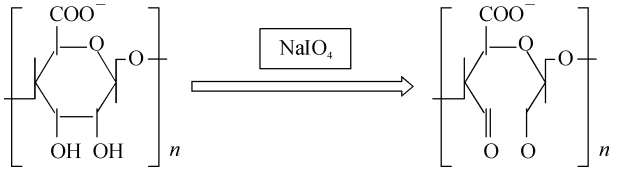


Fig. 1 The reaction scheme of A-SA

FTIR spectra of sodium alginate and A-SA (Fig. 2) showed raw sodium alginate had a lot of hydroxyl (Fig. 2 (a)), and the broad peak appearing at 3 300–3 800 cm^{-1} was attributed to hydroxyl on raw sodium alginate. Compared with the raw sodium alginate, the oxidized sodium alginate had a new absorption peak at 1 732 cm^{-1}, and it was attributed to aldehyde carbonyl (C═O) group of A-SA. Therefore, the A-SA generated the aldehyde group, thus further proved the possibility of the reaction mechanism in Fig. 1.

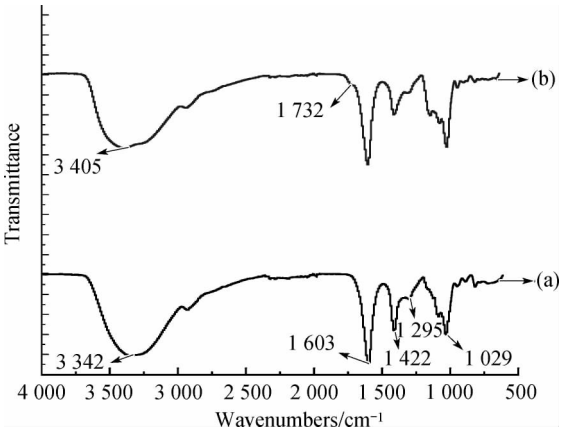
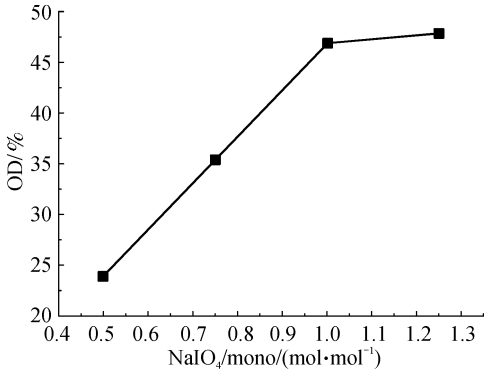


Fig. 2 FTIR spectra of (a) raw sodium alginate and (b) A-SA

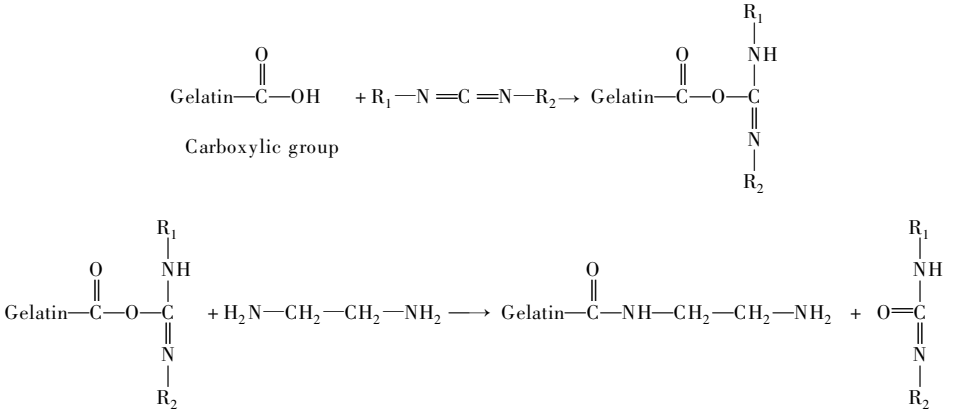
We used titration method [12-13] to measure the aldehyde content to confirm the OD of A-SA. The relationship between OD and oxidizer dosage ($NaIO_4$) was shown in Fig. 3, where the OD was increased with the oxidizer dosage increasing. When the molar ratio of $NaIO_4$ and monomer unit (repeating unit) of sodium alginate (mono) was higher than 1.0, the oxidation efficiency began to reduce.



ig. 3 The relationship between OD and $NaIO_4$/mono

2.2 The modified and characterization of amino-gelatin

The carboxyl groups (—COOH) in gelatin were converted into amino groups ($—NH_2$) by reaction with ED in the presence of EDC as reported [17-18] (Fig. 4).



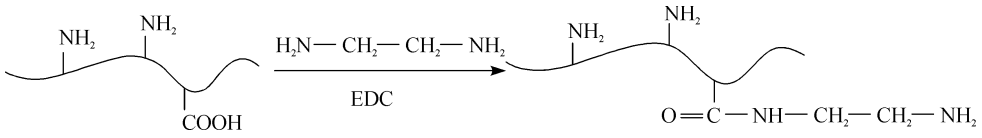


Fig. 4 The modified scheme of amino-gelatin

As shown in Fig. 5 (a), the absorption bands at about 3 320, 1 649, and 1 535 cm^{-1} were attributed to amine group and amide group of gelatin. The peaks occured in 2 868 and 2 939 cm^{-1} were methylene of amino acid. Compared with the raw gelatin, the absorption peaks of amino-gelatin at 1 535 and 1 649 cm^{-1} were enhanced, and this may be the results of ED reacting with raw gelatin to produce more amino group and methylene of amino-gelatin.

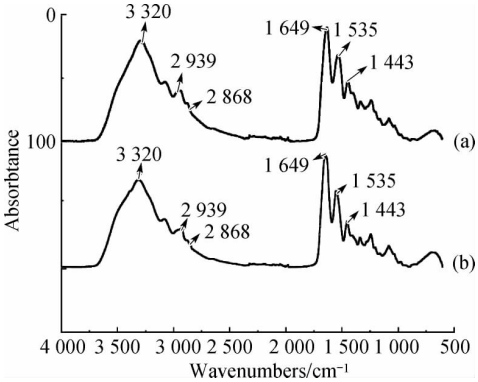


Fig. 5 FTIR spectra of (a) gelatin and (b) amino-gelatin

The amino group content in amino-gelatin was determined by the TNBS method described previously [8]. When the molar ratio of EDC and gelatin (—COOH content) was 2∶1, the relationship between $—NH_2$ content of amino-gelatin and the ED content was shown in Table 1. With the increasing of ED concentration, the introduced amino group content increased.

Table 1 The amino group content of amino-gelatin influence to the sol-gel temperature

Sample	[ED] / [COOH]	Amino content /%	Sol-gel temperature /℃
Gelatin	0	—	55
Amino-gelatin-1	1	22	40
Amino-gelatin-2	5	34	26
Amino-gelatin-3	20	55	24
Amino-gelatin-4	100	82	19

The vial-inverting approach was used to determine the sol-gel transition temperatures of gelatin and amino-gelatin solutions. Each sample with a given concentration was stored in vials at 4 ℃. Then the vials with polymer hydrogels were immersed in a water bath, and the samples were regarded as a "gel" in the case of no visual flow within 30 s by inverting the vial with a temperature increment of 1 ℃ per step [9-10]. The sol-gel temperature of amino-gelatin had improved significantly (Table 1).

2.3 The gelation time of A-SA and amino-gelatin

Figure 6 showed the Schiff's based reaction between aldehyde groups of A-SA and amino groups of amino-gelatin.

The absorption peak at 1 732 cm^{-1} of A-SA (Fig. 7 (a)) was aldehyde group. The bands at 1 649 and 1 535 cm^{-1} of amino-gelatin (Fig. 7 (b)) contributed to amino group and amide group. A strong absorption peak occured in 1 647 cm^{-1} of hydrogel (Fig. 7 (c)) belonged to C ═N. At the same

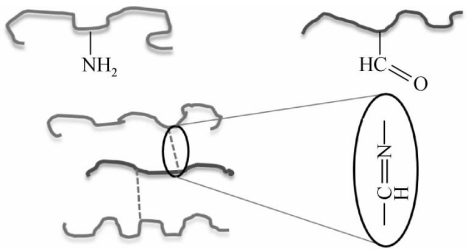


Fig. 6 Schiff's based reaction between amino group of amino-gelatin cross-linking and aldehyde group of A-SA

time, the absorption peak at 1 732 cm^{-1} of hydrogel (Fig. 7 (a)) decreased dramatically which meaned aldehyde group in hydrogel reduced. The aldehyde group decreasing and C ═N group arising indicated that the Schiff's base reaction occured in the hydrogel.

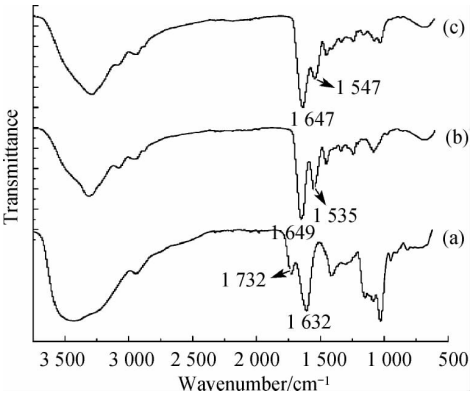


Fig. 7 FTIR spectra of (a) A-SA, (b) amino-gelatin, and (c) hydrogel

The gelation time of A-SA and amino-gelatin is controllable through changing the aldehyde group content in A-SA and amino group content in amino-gelatin. As shown in Fig. 8, the gelation time between the same OD of A-SA and different amino contents of amino-gelatin was measured at room temperature. With the amino content increasing in amino-gelatin, the gelation time cut down.

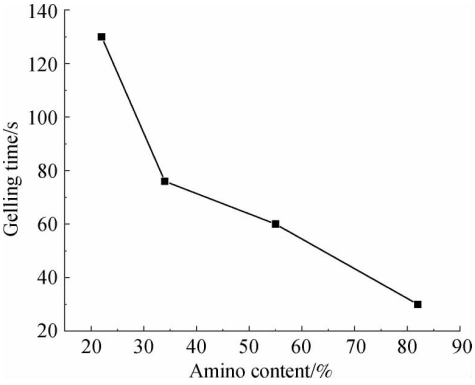


Fig. 8 The gelation time of the same A-SA (SA mono : $NaIO_4$ = 4∶3) and different amino contents of amino-gelatin at room temperature

3 Conclusions

The amino-gelatin had a lower sol-gel temperature compared with the raw gelatin. With different amino contents of amino-gelatin, the sol-gel temperature decreased from 55 to 19 ℃. Soft tissue adhesive hydrogels were successfully prepared by using A-SA and amino-gelatin. The gelation time was dependent on the aldehyde group content in A-SA and amino group content in amino-gelatin. The gelation time could be controlled within 30–130 s.

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