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RESEARCH ARTICLE

A soft tissue adhesive based on aldehyde-sodium alginate and amino-carboxymethyl chitosan preparation through the Schiff reaction

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ABSTRACT: Sodium alginate and carboxymethyl chitosan have been extensively applied in tissue engineering and other relative fields due to their low price and excellent 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). Carboxymethyl chitosan was modified with ethylenediamine (ED) in the presence of water-soluble N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) to introduce additional amino groups to get amino-carboxymethyl chitosan (A-CS). Upon mixing the A-SA and A-CS aqueous solutions together, a gel rapidly formed based on the Schiff's base reaction between aldehyde groups in A-SA and amino groups in A-CS. FTIR analysis confirmed the characteristic peak of Schiff's base group in the hydrogel. It was confirmed that the gelation time be dependent on the aldehyde group content in A-SA and amino group content in A-CS. The fasted hydrogel formation takes place within 10 min. The data of bonding strength and cytotoxicity measurement also showed that the hydrogel had good adhesion and biocompatibility. All these results support that this gel has the potential as soft tissue adhesive.

KEYWORDS: oxidized sodium alginate; amino-carboxymethyl chitosan; tissue adhesive; Schiff's base

Contents

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1 Introduction

In operating rooms, lacerations and disruption of skin, visceral organs, muscles, tendons, and ligaments are traditionally treated with sutures [1]. In the past few years, many researches were focused on polymeric adhesives which can be used for the closure and protection of wounds [2–7]. Broadly speaking, these adhesives have usually proven to be too toxic, too weak, or too short-lived to function effectively as soft adhesive [8]. It is necessary to create a kind of polymeric adhesives for wounds closure as soft tissue adhesive. Researchers have proposed that the ideal adhesive should satisfy the following conditions: strong and rapid adhesive, no or low immunogenicity, not expensive, biocompatible, facilitate storage, and biodegradable [9].

Hydrogels are a kind of materials that have been widely applied as tissue adhesives due to their three-dimensional (3D) polymeric networks which are similar to the structure of the native extracellular matrix (ECM) [10]. Because of that 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, the fluid balance in injury is very important [11]. In addition, soft tissue adhesive should have certain other properties such as the ease of application and removal, and proper adherence so that there will no area of non-adherence left to create fluid-filled pockets for the proliferation of bacteria [12]. A soft tissue adhesive can effectively stop bleeding by a simple procedure. It is a dynamic process and the performance requirements of a dressing can change during the healing progresses. However, it is generally accepted that a moist and warm environment encourages rapid healing, and a great mount of current wound care products are designed to provide these properties [13].

Alginate are anionic linear polysaccharides with 1,4-linked α-L-guluronate (G) and 1,4-linked β-D-mannuronate (M) residues in varying proportions. Carboxymethyl chitosan has many amino and carboxyl groups which provide modified site [14]. In this paper, the hydrogel was prepared by aldehyde-sodium alginate (A-SA) and amino-carboxymethyl chitosan (A-CS). Sodium alginate was partially oxidized by sodium periodate. Fourier transform infrared spectroscopy (FTIR) analysis confirmed the characteristic peak of aldehyde group in A-SA. Besides, the aldehyde contents (the oxidation degree) can be measured by using titration methods [15]. With the increasing amount of sodium periodate added, the aldehyde contents of A-SA increased. The amino contents of A-CS can be measured by using the trinitro-benzene-sulfonic acid (TNBS) method. Amino groups increase with the increasing amounts of ethylenediamine (ED) which is used to react with carboxymethyl chitosan. The introduction of amino groups in carboxymethyl chitosan improves its solubility and facilitates carboxymethyl chitosan dissolving in water at room temperature. Gelation occurs to form self crosslinking hydrogels as soft tissue adhesive by the Schiff's base between aldehyde groups of A-SA and amino groups of carboxymethyl chitosan. These two components can rapidly form a gel and be tightly bonded to soft tissue when mix. The gelation time is dependent on the content of the aldehyde groups in A-SA and amino groups in carboxymethyl chitosan. In this method, wound dressings which can be formed *in situ* improved many properties such as ease of application, compliance and comfort of patience.

2 Materials and methods

2.1 Materials

Sodium alginate (medium viscosity grade) received from Qingdao Mingyue Company, and carboxymethyl chitosan, sodium periodate, ED, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), and TNBS were purchased from Aladdin. All other chemicals are of reagent grade and used as received.

2.2 Preparation of A-SA

The modification of sodium alginate of aldehyde group was done according to the previous paper [12,16]. In brief, 5 g of sodium alginate was dissolved in 250 mL deionized water. Then different amounts of sodium periodate dissolved in 100 mL deionized water was dropwise added to sodium alginate solution (molar ratios of sodium alginate unit to $NaIO_4$ were respectively 4:4, 4:5, 4:6) without light. The mixture solution was then stirred at room

temperature for 4 h and kept out of the light. After that, 5 mL ethylene glycol was added to terminate the action. The result mixture solution was dialyzed (cut off Mw = 14000) for 3 d against deionized water, lyophilized to obtain the final product.

The aldehyde content of A-SA was measured by the titration method [14]. Briefly, mixed 5 mL hydroxylamine hydrochloride solution and 5 mL ethanol together, and then heated and refluxed the mixture for 10 min. Used 0.1 mol/L HCl aqueous solution to titrate the mixed solution. Stopped the titration and recorded the volume (V_2) of HCl aqueous solution used to titrate until the color of the solution became yellow. W g A-SA was dissolved in the mixed solution of 5 mL hydroxylamine hydrochloride solution and 5 mL ethanol. Repeat the step of titration and record the volume (V_1) of HCl aqueous solution. The oxidation degree can be calculated as follow:

$$\text{Oxidation degree}/\% = 198 \times 0.1 \times \frac{V_2/\text{mL} - V_1/\text{mL}}{2W/\text{g}} \times 100 \quad (1)$$

2.3 Preparation of A-CS

The method of modifying carboxymethyl chitosan was inspired by the amination of gelatin according to the previous paper [13]. In brief, 5 g carboxymethyl chitosan was dissolved in 200 mL (0.1 mol/L) sodium dihydrogen phosphate (NaH_2PO_4) solution (pH = 5). Specific amounts of ED and EDC sequentially added, and then the pH was adjusted back to 5 by hydrochloric acid. The molar ratio of carboxyl groups (COOH) on carboxymethyl chitosan chains to EDC and to ED was 1:2:20. The reaction was allowed to proceed at 37°C for 6 h. After that, the result product was dialyzed (cut off Mw = 14000) against deionized water for 3 d to remove the redundant ED and EDC, and the product was then frozen at −80°C, lyophilized and stored.

The amino group content in carboxymethyl chitosan was determined by the TNBS method [17]. Briefly, 0.1 g A-CS was dissolved in 10 mL phosphate buffered solution (PBS) to prepare 1% (w/v) A-CS solution. 1 mL 1% (w/v) A-CS was mixed with 1 mL 4% $NaHCO_3$ and 1 mL 0.1% (w/v) 2,4,6-trinitrobenzene-sulfonic acid in a brown tube. After incubation at 40°C for 2 h, the optical density (OD) of the solution was measured at 420 nm using Thermo Scientific Multiskan Go. The content of CS was measured by the same method. The result was converted into free amino content (mmol/g) using a calibration cure obtained with the β-alanine solution as a standard.

2.4 Gelation time measurement

0.5 mL of the A-SA solution of adhesive glue was dropped into a glass tube which was incubated at room temperature, and a 1 cm magnetic bar with the stirring speed of 2 r/s was put into the glass tube. Afterwards, 0.5 mL of the A-CS solution of adhesive glue was dropped into the tube and mixed with 0.5 mL of the A-SA solution. The time period required for the magnetic bar to stop was recorded as the gelation time of the adhesive glue.

2.5 Measurement of the swelling ratio

The swelling ratio of the prepared gels was obtained by the weighing method. In detail, the disc-shaped hydrogels were allowed to swell in 10 mL PBS (pH = 7.2) at 37°C in triplicate. After 24 h, the liquid on the surface of the samples was softly wiped off with a filter paper, and then the samples were weighed and the values of weight (W_s) were recorded. After the samples were dried in a vacuum oven at 37°C overnight, similarly the samples were weighed and the values of weight (W_d) were recorded. The swelling ratio was calculated as follow:

$$\text{Swelling ratio}/\% = \frac{W_s - W_d}{W_d} \times 100 \quad (2)$$

where W_s is the weight of the swelling equilibrium hydrogels, and W_d is the weight of dried hydrogels.

2.6 Bonding stress measurement

The fatty layer of porcine skin was removed using a scalpel and the fatty-layer-free porcine skin was sliced into 1 cm × 3 cm. 25 μL of one component solution of adhesive glue was put on the dermal side of each skin slice, and then the same volume of another component solution was mixed together on the skin slice. Two skins were then overlapped to a bonding area of 1 cm × 1 cm. After loading a weight of 50 g for 10 min, unless otherwise indicated, the bonding strength was measured via using a tensile machine at a testing rate of 10 mm/min [18].

2.7 Degradation study

The *in vitro* degradation experiment of the prepared

samples (n = 3) was performed at 37°C in the PBS (pH = 7.2) solution, and the degradation property was assessed by the percentage of weight loss. The time point that the experimental swollen hydrogels began to lose weight is the onset of degradation, and the weight of the swelling equilibrium hydrogels was denoted as W_o. After the PBS solution on the sample surface was removed at a predetermined time point, the samples were weighed and the values, set as W_t, were recorded. The samples were immersed into PBS in an incubator set at 37°C, and the PBS solution was refreshed at every point after the tested hydrogels were weighed. The weight loss ratio of the hydrogel samples was calculated as follow:

$$\text{Weight loss ratio}/\% = \frac{W_o - W_t}{W_o} \times 100 \qquad (3)$$

where W_o is the weight of the swelling equilibrium hydrogels when the hydrogels initially lost weight and W_t is the weight of the hydrogels at time t.

2.8 *In vitro* cell compatibility

The cytotoxicity of the hydrogels was evaluated by the MTT colorimetric method. The prepared hydrogels, which were formed in 24-well cell culture plate, were fumigated and sterilized in 75% ethanol vapor for 5 h, and then exposed in the ultraviolet (UV) light over night after washing 3 times with sterilized PBS. Mouse fibroblasts, L929, were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution under standard culture conditions at 37°C and 5% CO_2. The L929 cells were seeded into the hydrogels at a concentration of 10×10^4 cells per well. After 24 h incubation, the culture medium was removed and replaced with the DMEM supplemented with 10% FBS and 1% penicillin-streptomycin solution, and then incubated for other 72 and 144 h under the same conditions. 40 μL of 5 mg/mL MTT assay solution and 360 μL of DMEM were added to each well at a predetermined time after the culture medium was removed, and then returned back to incubate for 4 h. The medium containing unreacted MTT was removed. Then 200 mL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals, and after incubation for 30 min at 37°C, the optical absorbance values of the solutions were measured in an ELISA reader (Multiscan GO, Thermo Scientific) at a wavelength of 570 nm to determine the number of living cells.

3 Results and discussion

3.1 Characterization of A-SA

The preparation schematic of A-SA was shown in Fig. 1. The schematic diagram suggested that one monomer unit (or repeating unit) of sodium alginate can be opened to form two aldehyde groups by one periodate sodium. As shown in Fig. 2, FTIR spectra of sodium alginate and oxidized sodium alginate suggested that there are many hydroxyl groups on sodium alginate and oxidized sodium alginate according to the broad peak appearing at 3300–3800 cm^{-1}. Compared with the raw sodium alginate, there is a new absorbed peak appearing at 1732 cm^{-1} on the oxidized sodium alginate which is owing to the aldehyde carbonyl (C = O) group of oxidized sodium alginate. According to the analysis of FTIR, it is proven that the reaction is possibly attributed to the existence of aldehyde groups. The oxidation degree of A-SA is related to the molar ratio of sodium periodate to sodium alginate unit, $n(NaIO_4)/n$(SA unit), which is shown in Table 1.

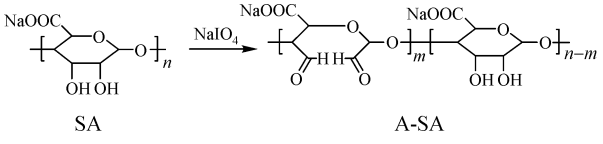

Fig. 1 The reaction scheme of A-SA.

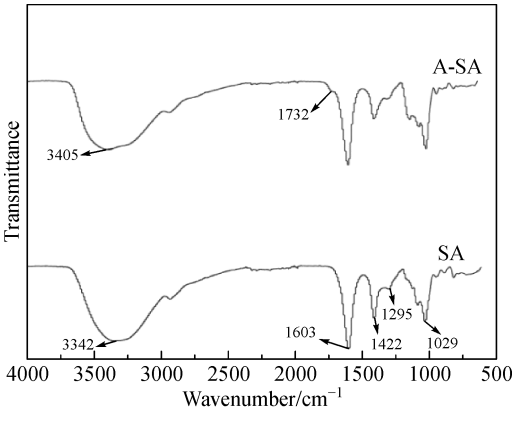

Fig. 2 FTIR spectra of SA and A-SA.

Table 1 The oxidation degrees of A-SA samples

Sample	$n(NaIO_4)/n$(SA unit)	Oxidation degree /%
SA	–	–
A-SA-1	1:1	53.56
A-SA-2	6:5	63.69
A-SA-3	3:2	75.24

3.2 Characterization of A-CS

The preparation schematic of A-CS was shown in Fig. 3. The schematic diagram suggested that with the existence of EDC, ED can react with the carboxyl groups on the CS chain to make more amino groups in the condition of pH = 5 [19–20]. This method can increase the amino groups on the carboxymethyl chitosan chain. Via the TNBS method, the amino content of A-CS was determined approximately as 0.0982 mmol/g, while that of CS was 0.0195 mmol/g. It was obviously that the amino group content of A-CS was much more than that of CS.

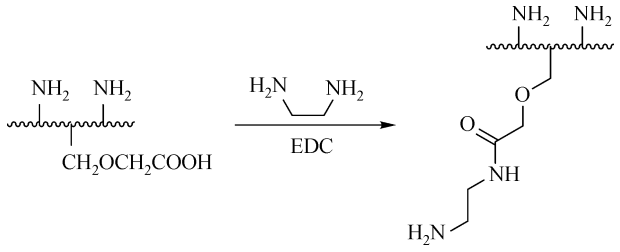

Fig. 3 The modification scheme of A-CS.

3.3 The gelation time of A-SA and A-CS

Figure 4 showed the Schiff's base reaction between aldehyde groups of A-SA and amino groups of A-CS. As shown in Fig. 5, the absorption peak at 1732 cm^{-1} of A-SA was aldehyde group. The band at 1649 and 1535 cm^{-1} was contributed to amino groups and amide group. A strong absorption occurred in 1647 cm^{-1} of hydrogel belonged to C = N. At the same time, the absorption peak at 1732 cm^{-1} of hydrogel decreased dramatically which meant aldehyde group in hydrogel reduced. The aldehyde group decreasing and C = N group arising indicated that the Schiff's base reaction occurred in the hydrogel.

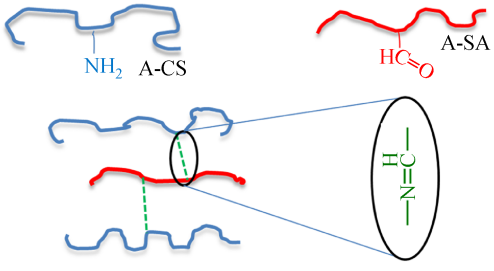

Fig. 4 The Schiff's base reaction between aldehyde group of A-SA crosslinking amino group of A-CS.

Gelation time was used to test how fast the two-component solutions can form a gel when they were mixed

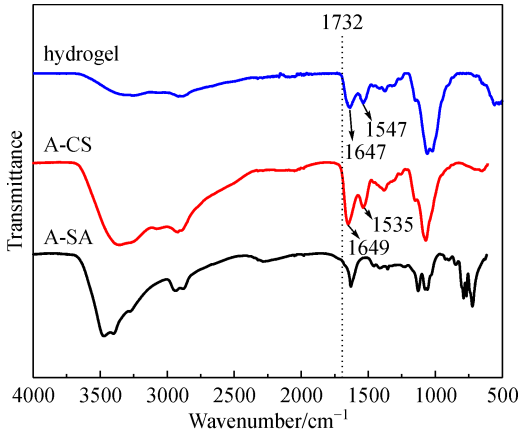

Fig. 5 FTIR spectra of A-SA, A-CS and hydrogel.

together, which can be used to evaluate the hemostasis of the hydrogel adhesive. As shown in Table 2, the A-SA-3/A-CS hydrogel was the fastest one to form a gel. It was obviously that the more aldehyde groups on A-SA and amino groups on A-CS, the faster hydrogels would form a gel. It was supposed that the mount of aldehyde groups and amino groups may influence the reaction speed.

Table 2 The gelation time of hydrogels

Sample	Gelation time /min
A-SA-3/A-CS	5–10
A-SA-2/A-CS	15–20
A-SA-1/A-CS	15–20
A-SA-3/CS	15–20
A-SA-2/CS	20–25
A-SA-1/CS	20–25

3.4 The swelling ratio of hydrogels

Swelling was the progress of water molecules that permeated into the inner hydrogels. From Fig. 6, we can see that the swelling ratio was decreased when the oxidation degree of sodium alginate was increased, and the swelling ratio of A-SA-3/A-CS hydrogels was the lowest. It was suggested that higher oxidation degree A-SA had more aldehyde groups to form a more compact web structure by reaction with amino groups, which can make water molecules hardly infiltrate into the inside of hydrogels.

3.5 The bonding stress of A-SA/A-CS hydrogels

The bonding stress of the hydrogel adhesive is represented by the lap shear strength of porcine skin adhered by hydrogels in this paper. As shown in Fig. 7, A-SA-3/A-CS

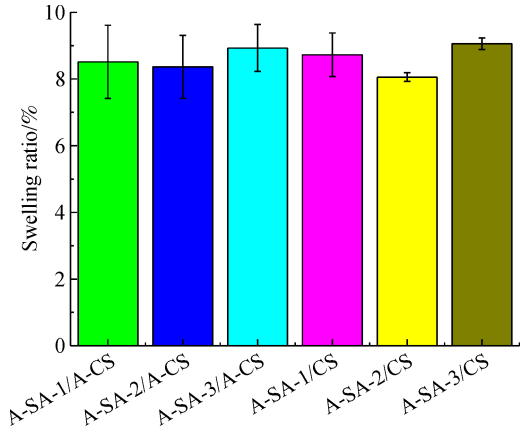

Fig. 6 The swelling ratios of hydrogels.

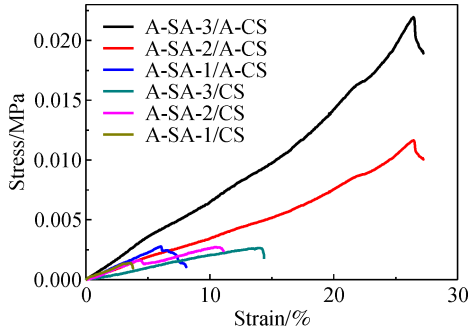

Fig. 7 The lap shear strength of porcine skin adhered by hydrogels.

hydrogels had the largest bonding stress ((0.0219±0.0034) MPa in Table 3) which is a little smaller than that of fibrin adhesive ((0.04953±0.0021) MPa). When the content of amino groups on A-CS or CS was constant, the bonding stress of the hydrogels was increased with the increase of the oxidation degree of A-SA. Similarly, when the oxidation degree of A-SA was constant, the bonding stress of hydrogels formed by A-CS was higher than that of those formed by CS. It was because that higher oxidized A-SA had more aldehyde groups, and A-CS had more amino groups than CS, which could form a more stable 3D crosslinking networks via the Schiff's base formed by aldehyde group and amino group.

3.6 The degradation of hydrogels

The degradation of hydrogels was estimated by the weight loss of hydrogels which were formed by mixing different oxidation degree A-SA with A-CS or CS. Figure 8 shows that the A-SA-3/A-CS hydrogels degraded more slowly in the PBS solution than the A-SA-2/A-CS and the A-SA-1/

Table 3 The max bonding stress and bonding strain of hydrogels

Sample	Max stress /MPa	Max strain /%
A-SA-3/A-CS	0.0219±0.0034	26.43±5.40
A-SA-2/A-CS	0.0098±0.0018	23.31±3.11
A-SA-1/A-CS	0.0024±0.0003	5.55±0.40
A-SA-3/CS	0.0022±0.0004	11.55±1.82
A-SA-2/CS	0.0033±0.0006	8.60±1.75
A-SA-1/CS	0.0015±0.0001	5.63±2.01

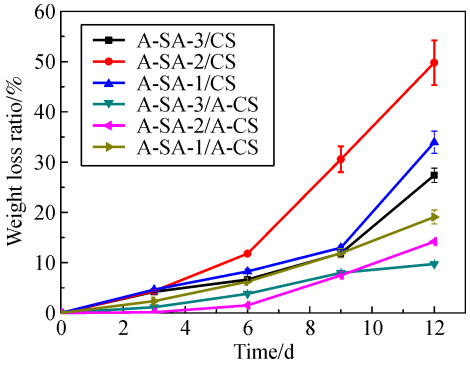

Fig. 8 The degradation of hydrogels.

A-CS hydrogels. When the oxidation degree of A-SA was constant, the hydrogels formed by A-CS degraded more slowly than those formed by CS. It is supposed that the hydrogels formed by higher oxidation degree A-SA and A-CS may have a more densified crosslinking structure, due to the more Schiff's base formed by aldehyde groups and amino groups, which is difficult to be degraded.

3.7 The biocompatibility of hydrogels

An ideal hydrogel adhesive should have a good biocompatibility and produce no harmful substance during the degradation. We can evaluate the biocompatibility of the hydrogel adhesives via the MTT measurement. As is shown in Fig. 9, the A-SA-3/A-CS hydrogels' OD value is better than TCP's and A-SA-3/CS. It is supposed that A-CS

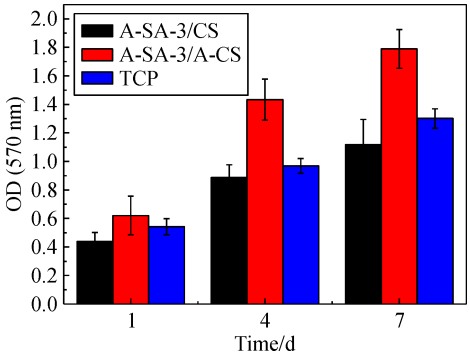

Fig. 9 The cell viability of hydrogels.

had more amino groups which can react with the aldehyde groups on the A-SA to form the Schiff's base and the amino groups are in favor of cell adhesion. Compared with that of A-SA-3/A-CS, the OD value of A-SA-3/CS is lower. This illustrated that A-SA-3/CS hydrogels had a little cytotoxicity because there were not enough amino groups on CS to react with aldehyde groups on A-SA-3 which is toxic to cells.

4 Conclusions

With the increased amount of sodium periodate, we can prepare higher oxidation degree A-SA which has more aldehyde groups. In addition, the amount of amino groups on CS was increased a lot by reacting with ED. More aldehyde groups can react with more amino groups, so that it can form a kind of adhesive which had a short gelation time and a stable crosslinking network. The bonding stress value of A-SA-3/A-CS hydrogel which is (0.0219± 0.0034) MPa is the highest one among all samples, and the bonding stress of hydrogel was related with the structure of hydrogel's crosslinking network. The swelling ratio of the hydrogel samples had no significant difference. In addition, the degradation experiment showed that the A-SA-3/A-CS hydrogel could provide a long-term 3D environment for cell proliferation due to its slow degradation. What's more, the hydrogel formed by aldehyde sodium alginate with the oxidation degree of 75.24% and A-CS had good biocompatibility. All these results suppose that this hydrogel has the potential to be used as soft tissue adhesive.

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