# Preparation and Characterization of Crosslinked Carboxymethyl Chitosan–Oxidized Sodium Alginate Hydrogels

**Li-Hong Fan,[1] Xiao-Ran Pan,[1] Yue Zhou,[1] Ling-Yun Chen,[2] Wei-Guo Xie,[3] Zhong-Heng Long,[3] Hua Zheng[1]**

[1]*Department of Chemical Engineering, Wuhan University of Technology, Wuhan 430072, China*
[2]*Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada*
[3]*The Third Hospital of Wuhan, 430060 Wuhan, China*

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**ABSTRACT:** Oxidized sodium alginate (OSA) was prepared through an oxidation reaction of sodium alginate in the presence of sodium periodate. *In situ* crosslinked hydrogels were obtained through the crosslinking reaction between the active aldehyde of OSA and the amino of the carboxymethyl chitosan (CMCS). The effects of the addition of OSA on the structure and properties of the hydrogels are discussed. The structures of OSA, CMCS, and the hydrogels were analyzed by Fourier transform infrared spectroscopy, and the morphology of the hydrogels was characterized by X-ray diffraction and scanning electron microscopy. The results from the gelation time test show that the hydrogels had the shortest gelation time of 6.3 s when the addition of OSA was 8 mL. The results from the swelling degree determination test showed that the hydrogel swelling degree first increased and then decreased with the addition of OSA. With the introduction of nanosilver, the hydrogels showed a degree of antibacterial performance. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2331–2337, 2011

**Key words:** oxidized sodium alginate; carboxymethyl chitosan; hydrogel; antibacterial effect

## INTRODUCTION

The naturally occurring polysaccharides sodium alginate (SA) and chitosan (CS) have received much attention in biomaterials for their excellent biocompatibility.[1] CS [β- (1→4)-linked 2-acetamido-2-deoxy-D-glucopyranose], a cationic biopolymer obtained through the *N*-deacetylation of chitin, is considered a nontoxic, biodegradable, biocompatible, and environmentally friendly material with many superior properties.[2–6] However, its poor solubility in water and in common organic solvents limits its widespread utilization. Carboxymethylation is a convenient way to convert CS into a water-soluble form. Carboxymethyl chitosan (CMCS) has a lot of unique chemical, physical, and biological properties, such as a low toxicity, biocompatibility, and a good ability to form films, fibers, and hydrogels;[7,8] therefore, it has been used extensively in many biomedical fields, such as a moisture-retention agents, bactericides, wound dressings, artificial bone and skin, blood anticoagulants, and components in drug-delivery matrices.[9–11]

Alginate, a water-soluble linear polymer obtained from brown algae, consists of (1-4)-β-D-mannuronic acid (M) and (1-4)-α-L-guluronic acid (G) units in the form of homopolymeric (MM or GG blocks) and heteropolymeric sequences (MG or GM blocks).[12,13] With their gelling ability, stabilizing properties, and high viscosity in aqueous solutions, alginates and their derivatives are widely used in the food, cosmetics, and pharmaceutical industries.[14–16] In addition, oxidized alginates present more reactive groups and a faster degradation when they are used in supports for controlled drug delivery.[17,18]

Silver metal and silver ions have been known to be effective antimicrobial agents for a long time.

*Correspondence to:* L.-H. Fan (lihongfan2000@hotmail.com).

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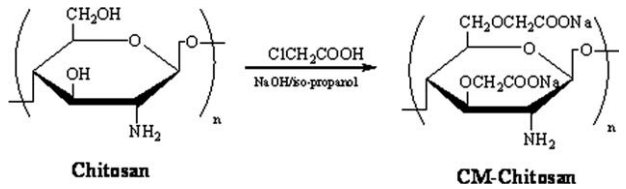



**Scheme 1** Carboxymethylation of CS.

There have been several kinds of silver-containing materials. For example, silver sulfadiazine containing CS-based wound dressings,[19–21] dendrimer-silver complexes and nanocomposites,[22] nanosilver/cellulose acetate composite fibers, and silver nylon dressings have all been proven to be antibacterial.[23,24]

Hydrogels consist of three-dimensional hydrophilic polymer networks in which a large amount of water is interposed. Because of their unique properties, a wide range of medical, pharmaceutical, and prosthetic applications have been proposed for them.[14] The introduction of nanosilver to a hydrogel will make it have antimicrobial properties so as to expand its application.

However, hydrogels based on oxidized sodium alginate (OSA) blended with CMCS have never been reported. We thought it would be interesting to study the preparation of OSA–CMCS hydrogels by an easy method. In this study, we aimed to prepare an OSA–CMCS hydrogel with the purpose of creating new wound dressing materials. In this study, we examined the structure, swelling degree, and antibacterial properties of the hydrogels. The effects of the addition of OSA on the structure and properties of the hydrogels are also discussed.

## EXPERIMENTAL

### Materials

CS was purchased from Zhejiang Yuhuan Ocean Biochemistry Co., Ltd. (Zhejiang, China). SA was purchased from Fuchen Tianjing Chemical Co., Ltd. (Tianjing, China). All other chemicals were reagent grade.

### Carboxymethylation of CS

Two reaction reagents, glyoxylic acid and chloroacetic acid, were used to prepare CMCS. The chloroacetic acid method was selected in this experiment.[25] In this work, CS was suspended in a solution of NaOH. The mixture was kept at −20°C overnight. Isopropyl alcohol was then added to the frozen alkali CS as a reaction medium; this was followed by the addition of chloroacetic acid. After it was stirred at room temperature for some time, the reaction mixture was heated to a temperature of 30°C for another 6 h. The final products were washed with ethanol and then vacuum-dried at 40°C (Scheme 1).

### Oxidation of SA

OSA was prepared according to a previously reported method.[26] Briefly, SA was dispersed in 100 mL of ethanol. An aqueous solution of sodium periodate was then added to the reaction mixture to get oxidized alginates. After it was stirred for some time in the dark at 30°C, the reaction was stopped by the addition of ethylene glycol and NaCl under stirring for 0.5 h. The final products were washed with ethanol and then vacuum-dried at 40°C. (Scheme 2).

### Preparation of nanosilver

Nanosilver was prepared according to a previously reported method.[27] $AgNO_3$ (0.018 g) was dissolved in water (100 g), and the aqueous solution was then heated to boiling. Subsequently, 2 mL of 1% sodium citrate was added to the solution. The mixture was kept boiling for 1 h to obtain a nanosilver dispersion.

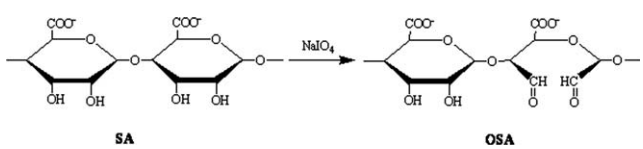
### Preparation of the CMCS–OSA hydrogel

CMCS (15 mL, weight = 6%) was mixed with OSA (2, 4, 6, 8, and 10 mL, weight = 15%). The mixture stood for some time to get the CMCS–OSA hydrogel. According to the amount of OSA, the hydrogels were marked as ASCS-2, ASCS-4, ASCS-6, ASCS-8, and ASCS-10, respectively.

Volumes of 0.5, 1, 1.5, 2, 2.5, and 3 mL of nanosilver solution were added to the CMCS solution; then, 6 mL of OSA solution was added to the mixture. The mixture stood for some time to get the CMCS–OSA hydrogel with nanosilver. According to the addition of nanosilver, the hydrogels were marked as ASCS-6$Ag_{0.5}$, ASCS-6$Ag_1$, ASCS-6$Ag_{1.5}$, ASCS-6$Ag_2$, ASCS-6$Ag_{2.5}$, and ASCS-6$Ag_3$, respectively.

### Gelation time

The gelation time test was performed according to a previously reported method.[28] A mixture of CMCS (weight ratio = 6%) and OSA solution (weight ratio = 15%) was put on a Petri dish (100 × 20 $mm^2$, VWR International), and a magnetic stirring bar (Teflon fluorocarbon resin, 5 × 2 $mm^2$, Fisher Scientific) was placed in the center of the solution droplet. The solution was stirred at 155 rpm with a Corning model PC-320 hot




**Scheme 2** Oxidation of SA.

plate/stirrer under UV illumination from a distance of 2 cm. The gelation time was decided when the solution formed a solid globule that completely separated from the bottom of the dish. The results are reported as averages and standard deviations of five independent measurements per gel.

### Swelling degree determination

The hydrogels were dipped in normal saline, a water solution of 0.9% of NaCl, at 37°C for 24 h. The hydrogel swelling degrees at equilibrium status (SDs) were calculated with the following equation:

$$\mathrm{SD} = (W_e - W_o)/W_o$$

where $W_e$ is the equilibrium weight of the hydrogel in normal saline and $W_o$ is the absolute dried weight of hydrogel.[29] The normal saline was used in our experiment was freshly prepared.

### Characterization

Fourier transform infrared (FTIR) spectra were recorded in a KBr disk with a Nicolet 170SX FTIR spectrometer (Perkin Elmer Co., USA) equipped with a Dynamic Ground Target Simulator (DGTS) detector and DMNIC 3.2 software over the range 4000–400 $cm^{-1}$. The X-ray diffraction (XRD) pattern of the hydrogels was recorded at room temperature with a Shimadzu Labx-XRD-6000 X-ray diffractometer (Shimadzu Corp., Japan). The surface and the cross-sectional morphology of the hydrogel were observed with a scanning electron microscope (Hitachi S-570, Japan).

### Determination of the hydrogel antibacterial properties

The *in vitro* antimicrobial activities of the hydrogels were determined by the Kirby–Bauer disc diffusion method.[30] The antibacterial activities of the CMCS–oxidized sodium hydrogels were determined against Staphylococcus aureus (ATCC 25923) and Colon Bacillus (ATCC 25922) with a nutrient agar method. The agar (3.2 g) was added to 100 mL of distilled water and autoclaved. Agar medium (10 mL) was then poured onto Petri plates and air-dried. The assay plates were then seeded with 0.5 mL of bacteria solutions of *S. aureus* and *E. coli*, respectively. After 4 h of incubation at 37°C, the slices of hydrogels were put into the agar plates. All of the plates were incubated at 37°C for 48 h. The diameters of any bacterial inhibition zones that had formed around the samples were measured with calipers. The inhibition zone diameter ($\Delta d$) of the hydrogel was calculated as follows:

$$\Delta d = D - d$$

where $D$ is the diameter of the outer edge of the inhibition zone and $d$ is the diameter of the hydrogel samples.

**TABLE I**
**Gelation Time and Swelling Degree of the Hydrogels**

| Gel | $W_o$ | $W_e$ | SD (%) | Gelation time (s) |
|---|---|---|---|---|
| ASCS-2 | 3.0125 | 8.0075 | 165.8 | 235.9 |
| ASCS-4 | 2.2553 | 8.0303 | 256.1 | 122.1 |
| ASCS-6 | 3.5892 | 10.6228 | 196.0 | 41.8 |
| ASCS-8 | 3.1713 | 9.2445 | 191.5 | 6.3 |
| ASCS-10 | 4.4112 | 8.4664 | 91.9 | 20.6 |
| ASCS-$6_{Ag1}$ | 3.6298 | 10.6820 | 194.3 | 52.6 |
| ASCS-$6_{Ag2}$ | 4.3235 | 12.8278 | 196.7 | 55.2 |
| ASCS-$6_{Ag3}$ | 3.0056 | 8.8936 | 195.9 | 68.5 |

## RESULTS AND DISCUSSION

### Gelation time

Different amounts of OSA were added to form different CMCS–OSA hydrogels. Different amounts of nanosilver were added to form different CMCS–OSA hydrogels with nanosilver. The gelation time results of the hydrogels in listed in Table I.

The gelation time of the hydrogels decreased from 235.9 to 6.3 s with the amount of OSA increasing from 2 to 8 mL. Then, the gelation time decreased to 20.6 s when the OSA amount increased to 10 mL. That was because at OSA amounts lower than 8 mL, the addition of OSA provided more aldehyde groups to react with the aminos of CMCS to form the crosslinked network structure, so the gelation time increased. Whereas when the amount of OSA was more than 8 mL, aldehydes in the solution became saturated; with the addition of OSA aqueous solution, the concentration of the whole solution was reduced, which impeded aldehyde groups from reacting with aminos to form the crosslinked network structure. This resulted in longer times for hydrogel gelation. When the amount of OSA was 8 mL, gelation of the gel reached the maximum speed, which was within 6.3 s. The extremely short time of gelation provided sufficient conditions for the hydrogels to be used in the medical field.

From the gelation time data of the CMCS hydrogels with different amounts of nanosilver, we found that with the increase of nanosilver, the gelation time of the hydrogels became longer. The reason was that the addition of nanosilver solution reduced the concentration of the whole system, which impeded the formation of the crosslinked network structure of the hydrogels and resulted in a longer gelation time of the hydrogels.

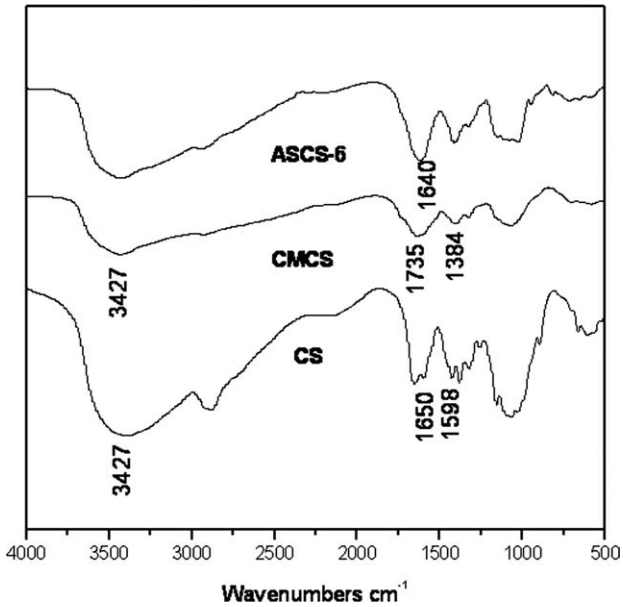
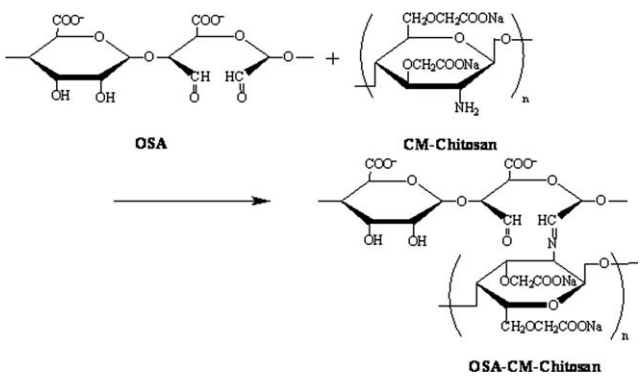



**Figure 1** FTIR spectra of CS, CMCS, and ASCS-6.

## Hydrogel swelling degree

Different CMCS–OSA hydrogels were formed according to the addition of OSA. The swelling degree results are shown in Table I. An obvious swelling behavior was observed for the hydrogels. The swelling degree was significantly influenced by the amount of OSA. With the increase of OSA, the hydrogel swelling degree increased at first and then decreased. With the addition of OSA, the network structure of the hydrogels became stable, so the swelling degree increased, but the addition of OSA also provided more aldehyde groups to crosslink with the aminos of CMCS. This made the structure of the hydrogels more and more compact, so the swelling degree decreased. When the amount of OSA was 4 mL, the swelling degree of the gel reached the maximum (256.1%). The introduction of antimicrobial agents had no significant effect on the swelling degree of the hydrogels.




**Scheme 3** Schematic illustration of the synthesis route of the hydrogel.

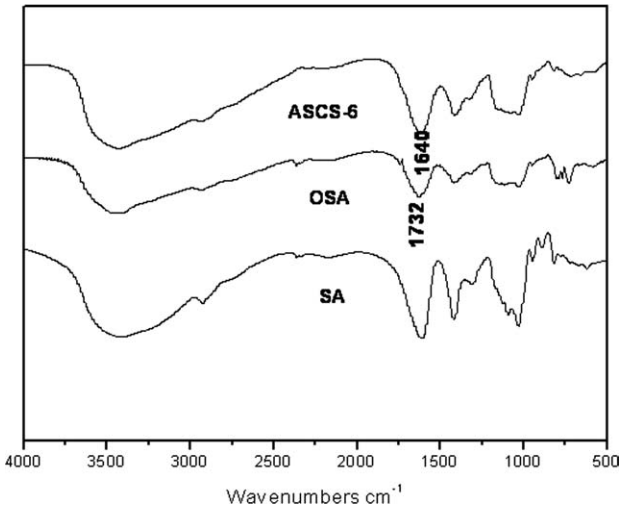



**Figure 2** FTIR spectra of SA, OSA, and ASCS-6.

## Infrared spectra analysis of the hydrogels

Figure 1 shows the infrared spectra of the CS, CMCS, and CMCS hydrogel (ASCS-6). ASCS-6 was the CMCS hydrogel formed by CMCS and 6 mL of OSA. From the infrared spectra of CS, we could see an absorption peak at 3427 $cm^{-1}$, which was due to the O—H stretching vibration, N—H extension vibration, and intermolecular H bonds of the polysaccharide moieties.[31] The peak at 1598 $cm^{-1}$ was attributed to the deformation vibration of amido, and that at 1650 $cm^{-1}$ was assigned to amide stretching vibrations.[14] From the infrared spectra of CMCS, we could see a strong new peak at 1735 $cm^{-1}$ representing the carboxylate C=O asymmetric stretching. The peak at 1384 $cm^{-1}$ was assigned to the symmetric stretching vibration of carboxylate C=O;[31] this demonstrated the introduction of —COO group to CS chains. Compared to the

**TABLE II**
**$\Delta d$ of ASCS-$6_{Ag}$ in *S. aureus* (SA) and Colon Bacillus (CB)**

| Gel | $d_{SA}$ (mm) | $D_{SA}$ (mm) | $BRR_{SA}$ (%) | $d_{CB}$ (mm) | $D_{CB}$ (mm) | $BRR_{CB}$ (%) |
|---|---|---|---|---|---|---|
| ASCS-6 | 0.9 | 1.1 | 22.2 | 1 | 1.1 | 10 |
| ASCS-$6_{Ag0.5}$ | 0.8 | 2.5 | 212.5 | 0.9 | 2.5 | 177.8 |
| ASCS-$6_{Ag1}$ | 1 | 3.5 | 250 | 0.8 | 2.6 | 225 |
| ASCS-$6_{Ag1.5}$ | 1 | 3.9 | 290 | 0.8 | 2.7 | 237.5 |
| ASCS-$6_{Ag2}$ | 1 | 4.3 | 330 | 0.8 | 2.9 | 262.5 |
| ASCS-$6_{Ag2.5}$ | 0.9 | 4.1 | 355.6 | 1 | 3.8 | 280 |
| ASCS-$6_{Ag3}$ | 0.8 | 4 | 400 | 0.9 | 3.9 | 333.3 |

$d_{SA}$, diameter of hydrogel samples against Staphylococcus aureus; $D_{SA}$, diameter of the outer edge of the inhibition zone against Staphylococcus aureus; $BRR_{SA}$, antibacterial ratio against Staphylococcus aureus; $d_{CB}$, diameter of hydrogel samples against Colon Bacillus; $D_{CB}$ diameter of the outer edge of the inhibition zone against Colon Bacillus; $BRR_{CB}$ antibacterial ratio against Colon Bacillus.

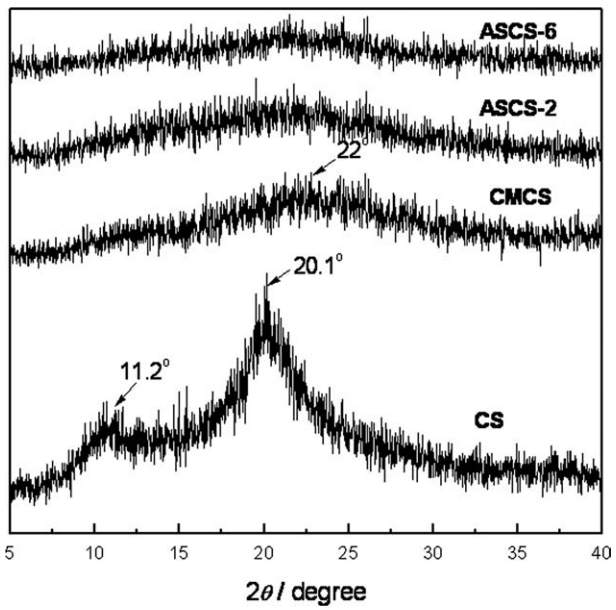



**Figure 3** XRD patterns of CS, CMCS, ASCS-2, and ASCS-6.

infrared spectra of CS, the absorption peak of CMCS at about 3427 $cm^{-1}$ became weak; this indicated the O—H of CS participated in the reaction (as the absorption peak at 3427 $cm^{-1}$ was due to the O—H stretching vibration).

Compared to the infrared spectra of CMCS, the CMCS hydrogel had a new absorption peak at 1640 $cm^{-1}$. It was attributed to the —N=C— stretching vibration peak,[32] produced by the Schiff base reaction between the amino groups in CMCS and aldehyde groups in OSA (Scheme 3). The occurrence of —N=C— proved that the crosslink reaction had taken place.

Figure 2 shows the infrared spectra of the SA, OSA, and CMCS hydrogel (ASCS-6). Compared to the infrared spectra of SA, OSA had a new absorption peak at 1732 $cm^{-1}$; it could be attributed to the stretching vibration of —CHO,[33] which showed that the oxidation reaction had taken place. The peak disappeared in the infrared spectra of the CMCS hydrogel, which indicated the aldehyde group of OSA was consumed in the crosslink reaction.

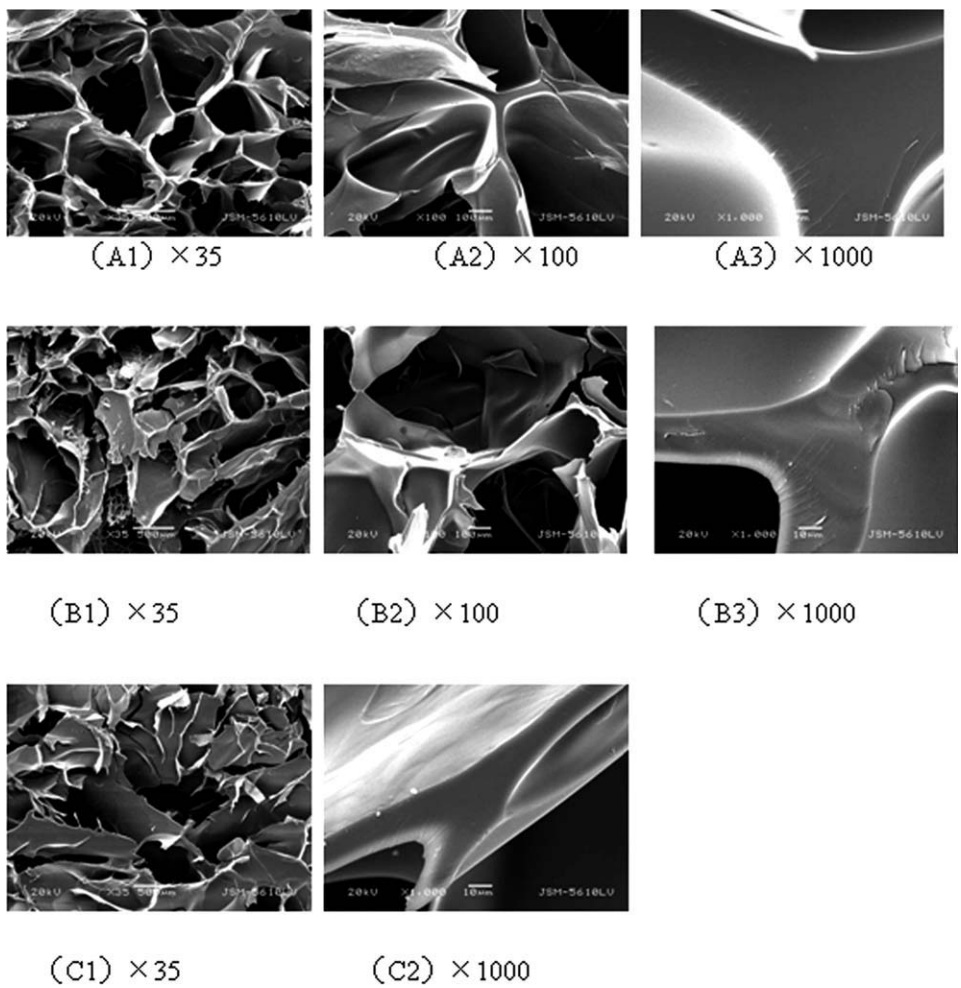



**Figure 4** SEM images of (A) ASCS-2, (B) ASCS-10, and (C) ASCS-$6_{Ag3}$.

### Antimicrobial hydrogel analysis

Table II shows the inhibition zone data of ASCS-6 containing different amounts of nanosilver against Colon Bacillus and Staphylococcus aureus. ASCS-6 without nanosilver had weak inhibition against bacteria, which was because CMCS itself had a certain degree of inhibition against bacteria.[34] The antibacterial activity of the samples were largely enhanced with the introduction of the antibacterial agent. From the data, we could also see that the antibacterial properties of the hydrogels increased with the increase of nanosilver.

### XRD

XRD patterns of CS, CMCS, and CMCS hydrogels with different amount of OSA (ASCS-2 and ASCS-6) are shown in Figure 3. The strong crystalline diffraction peaks of CS at 20.1 and 11.2° disappeared after the carboxymethylation reaction. During the progress of the carboxymethylation reaction, the crystalline structure of CS was completely destroyed and turned into an amorphous structure. The weak peak of CMCS at 22° disappeared after crosslinking with OSA. This was due to the crosslinking reaction between the amido group in CMCS and the double aldehyde in the OSA (Schiff base reaction), which destroyed the crystal structure of the CMCS and resulted in the disappearance of the diffraction peaks.

### Scanning electronic microscopy (SEM)

Figure 4 shows SEM photographs of the CMCS hydrogels, showing their morphology and internal space of the three-dimensional network structure. The infrared spectra and XRD results show that the amido on CMCS and the double aldehyde on OSA were crosslinked. From these electron microscope photographs, we could see that the crosslinked hydrogels formed a stable network structure.

Images of ASCS-2 and ASCS-10 at different magnifications are shown in Figure 4(A,B). As shown by a comparison of Figure 4(A1) with Figure 4(B1), the internal spatial structure of the hydrogel with a smaller amount of OSA was much looser. Figure 4(A3,B3) shows that the hydrogel had a smooth structure on its surface; the addition of OSA had no other obvious impact on the microstructure of the hydrogel.

Images of ASCS-$6_{Ag3}$ at different magnifications are shown in Figure 4(C). Compared to the SEM photographs in Figure 4(A,B), which are two sets of blank hydrogels without nanosilver, we found that the introduction of antimicrobial agents had no significant effect on the three-dimensional network structure of the hydrogel.

## CONCLUSIONS

In this study, we obtained OSA through the oxidation reaction of SA in the presence of sodium periodate. Hydrogels were obtained through the crosslinking reaction between the active aldehyde of OSA and the amino of the CMCS. The shortest possible time for gelation of the hydrogel was 6.3 s, when the added amount of OSA was 8 mL. The swelling degree of the hydrogel increased at first and then decreased with the increase of OSA; when the added amount of OSA was 4 mL, the swelling degree of the gel reached the maximum (256.1%). The swelling properties of the hydrogel and its extremely short time for crosslinking provided sufficient conditions for it to be used in the medical field as an *in situ* crosslinked hydrogel.

We introduced nanosilver as an antimicrobial agent into the hydrogels and used Colon Bacillus and Staphylococcus aureus strains in the experiment to test the antibacterial properties of the hydrogels. The results show that the introduction of nanosilver significantly improved the antibacterial properties of the hydrogels.

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