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# The Synthesis of Nano-silver/Sodium Alginate Composites and Their Antibacterial Properties

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Running Title: Antibacterial properties of nano-silver/sodium alginate composites

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**ABSTRACT**

Nano-silver/sodium alginate (Ag/SA) composites were synthesized by an effective strategy. Sodium alginate (SA) solution was mixed with silver nitrate solution and heated under normal pressure to afford Ag/SA by in-situ reaction of liquid phase where SA acted as both reducing and stabilizing agents. The developed Ag/SA composites were characterized using UV-visible, XRD, TEM and TGA to understand their physical and chemical properties. These Ag/SA composites showed antibacterial activity towards *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

*Keywords*: silver nanoparticle; alginate; composite; antibacterial activity

## 1. Introduction

Compared with a bulk solid, nano-sized silver particles exhibit excellent electric,[1,2] optical[3] and catalytic properties[4,5] due to their large surface area and high reactivity. Furthermore, Silver nanoparticles are well-known to be excellent hygienic and curative agents because of their broad-spectrum antimicrobial activity and limited microbial resistance.[6] They have been used as antimicrobial agents, for the treatment of wounds and burns and even marketed as a water disinfectant or room spray.[7,8]

However, in order to fully enhance the antibacterial properties of silver nanoparticles, they are usually dispersed on the surface of the polymer matrix without the formation of large aggregates, which otherwise dramatically reduce the antimicrobial effect of silver.[9] More recently, great interest has been focused on developing nano-silver/polymers composites. Shi et al. fabricated composite membranes consisting of poly(tetrafluoroethylene), polypyrrole and silver nanoparticles.[10] The dispersibility of nano-silver was increased observably through the method of silver nanoparticles adsorbing on the polypyrrole, and the composite membrances exhibited excellent antibacterial properties. Goli et al. synthesized the polypropylene fibers whose surfaces were coated with silver nanoparticles. The Ag/polypropylene fibers composites exhibited excellent antibacterial activity with 100% removal efficiency.[11] Nevertheless, most of the synthetic polymers are nondegradable and the monomers of the polymers are toxic, which restricts the utilization of nano-silver/polymer composites. With increasing concern over microbial infections, there is a growing demand for effective and safe antimicrobial agents.[12,13] Sodium alginate (SA) is a linear anionic natural polysaccharide composing of 1,4-linked resiues of β-D-dmannuronic (M) acid and α-L-guluronic acid (G).[14] Because of its security, nontoxicity, biocompatibility and biodegradability, SA has been applied in many fields such as pharmaceutical,[15] textile[16] and food[17] applications.

To combine the advantages of nano-silver and SA, herein, the Ag/SA composites were synthesized through in-situ reaction of liquid phase where SA acted as both reducing and stabilizing agents. We explored an effective method, which restrained the aggregation of nano-silver and prepared the Ag/SA composites. Furthermore, we investigated the antibacterial properties of Ag/SA. This study may be used to access the possibility of the Ag/SA as potential materials for biomedical applications.

## 2. Materials and methods

### 2.1 Materials

Sodium alginate (SA, $\overline{M}_\eta$~ 430 kDa, M/G = 0.18), silver nitrate and absolute ethanol were bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Distilled water was used for the preparation of all

solutions. Chemicals for bacteriostatic experiment were provided by College of Bioscience and Biotechnology Yangzhou University.

**2.2 Synthesis of Ag/SA**

The preparation of Ag/SA was simply achieved by the reduction of $AgNO_3$ with SA in aqueous solution. 1, 2, 3 and 4mL of 0.04M $AgNO_3$ and 20mL of 10mg/mL SA solutions were first mixed separately at room temperature to give clear solutions. Reaction between $AgNO_3$ and SA was carried out under $N_2$ atmosphere at 100□ for 3h. The mixture was allowed to cool in air to ambient temperature and then dialyzed against the distilled water for 4 days to remove the free ions. After being concentrated to 20mL, the concentrated liquor was stored at 4□. In addition, to determine the amount of silver nanoparticels in the Ag/SA composites, the concentrated liquor was centrifuged and then washed with deionized water. Finally the silver nanoparticles were dried and weighted in $N_2$ atmosphere.

**2.3 Characterization**

To confirm the formation of nano-silver in SA solution, UV-vis measurements were performed on a UV-vis spectrophotometer (UV-vis, 2501, Japan) in the frequency interval of 200-800nm. Transmission electron microscopy (TEM, Tecnai-12, Holland) was used to research the morphology and size of synthetic nanosilver. X-ray diffraction was used, with a D8 Advance Superspeed X-ray diffraction (X-ray, Germany) with Cu as anode target ($\lambda$=0.15406nm), pipe pressure of 40kV, flow of 200mA to analyse the phase composition of samples. The measurements were carried out in the 2$\theta$ range of 30°-80°. Thermo gravimetric analysis (TG, STA409PC, Germany) was used to research the stability of SA and Ag/SA.

**2.4 Antibacterial properties and determination of minimal inhibitory concentrations**

The antibacterial properties of the Ag/SA against *Gram-negative Escherichia coli (E. coli* ATCC 25922*)* and Gram-positive *Staphylococcus aureus (S. aureus* ATCC 25923*)* were evaluated. The minimal inhibitory concentrations (MICs) of the Ag/SA were determined by the method of the broth microdilution in a microtiter plate.[18] These tests were performed in Müller-Hinton (MH) broth for the bacterial strains. The MH broth cultures were prepared for the experiments and the final concentration of the bacteria in every well was adjusted to $5\times10^5$ CFU/mL. 100uL of Ag/SA composites solution including 0.16mg/mL nano-silver was added in the first column, then serial doubling dilutions were prepared in MH broth for bacteria. In the experiments, the bacteria were fostered at 37□for 24h. The MICs were defined as the lowest concentration of the Ag where the well was pellucid.

**2.5 Antibacterial properties of cotton fabrics treated with the Ag/SA**

Antibacterial properties of the cotton fabrics containing Ag/SA against *S. aureus* ATCC 25923 were tested. The cotton fabrics with 15mm diameter were disinfected overnight under the UV lamp. 0.1mL of Ag/SA composites solution was dripped on the cotton fabrics to make sure the cotton fabrics could adsorb the Ag/SA composites solution completely. The cotton fabrics were dried at room temperature in aseptic conditions. Blank cotton fabrics and cotton fabrics containing Ag/SA composites were placed in separate positions on Muller Hinton Agar (MHA) plates in aseptic conditions. To make sure cotton fabrics were sandwiched between the MHA layer, another thin layer of MHA was poured onto the fabrics. 100uL of bacterial solutions of *S. aureus* ATCC 25923 ($5\times10^5$ CFU/mL) were inoculated on to MHA plates and evenly spread. The inoculated agar plates were incubated at 37 □ for 24h. In order to research the washing resistance of the cotton fabrics whose surfaces were coated with the Ag/SA composites, cotton fabrics dripped with the Ag/SA composites solution were dipped in saturated solution of calcium chloride for 30min, dried in aseptic conditions, washed a certain number of times, then repeated the bacterial experiments.

## 3. Results and discussion

### 3.1 Formation of silver nanoparticles

The prepared Ag/SA composites were characterized by UV-visible spectroscopy. Fig. 1a depicted the UV-vis spectrum of silver nanoparticles solution. The absorption spectra of sample showed a characteristic surface plasmon absorption band 410nm, which indicated the presence of nano-silver in the solution.[19] These results revealed that the SA reduced the silver ions into nano-silver successfully under heating conditions. The solution of Ag/SA composites (0.16mg/mL nano-siver) were placed onto copper grill and then dried at room temperature, finally, examined using TEM. As shown in Fig. 1b, the synthesized silver nanoparticles with aspheric shapes were dispersed in SA matrix and the sizes of it ranged from 20 to 30nm. Fig. 1c was the X-ray diffraction (XRD) spectra for synthetic nano-silver. The five diffraction peaks could be indexed to diffraction from the (111), (200), (220), (311) and (222) of face-centered cubic (fcc) Ag, which were the same with that of Ag on the card (JCPDS Card File, 4-0783). By the XRD analysis, it was shown that the crystal structure of nano-silver was fcc and belonged to Ag.

SA which has certain space structure was used as a template, and silver ions could be distributed in the template. Because of the strong interactions between silver ions and the hydroxyl and carboxyl groups of SA macromolecule, silver ions were uniformly and tightly anchored to the SA. Such interactions would lower the mobility of silver ions, enhance the formation of silver nuclei, and prevent the growth of larger particles. The hydroxyl of SA would reduce the silver ions into nano-silver packaged by SA under heating conditions. [20,21]

### 3.2 Thermostability of Ag/SA compoistes

Thermograms of SA and Ag/SA were demonstrated in Fig. 2. As we know, the first stage region belonged to the loss of combined water of SA.[22] In the second stage, the loss of weight was due to the thermal scission of the carboxylate groups and the evolution of $CO_2$ from the corresponding carbohydrate backbone. The weight loss of SA in the second stage began at 170□. However, in Ag/SA system, the second stage of the decomposition temperature improved to 220□. So we could suspect that the thermal stability of the Ag/SA composites was obviously higher than SA.

### 3.3. Antimicrobial properties

The antibacterial and fungicidal properties are an important characteristic of any material that is intended for biomedical applications. Ag-based compounds are highly toxic to microorganisms and they show strong germicidal effects on at least 12 kinds of bacteria.[23] As shown in Fig. 3, the SA couldn't restrain the growth of the bacteria, but the as-synthesized Ag/SA composites possessed the antimicrobial properties. The MICs for the synthesized Ag/SA composites against the *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were 0.25 and 0.13μg/mL separately (Table 1). Compared the MICs value with the MICs for nano-silver (0.78-6.25μg/mL)[9] and pluronic-coated silver nanoprisms (24μg/mL),[24] the Ag/SA composites exhibited superior antimicrobial properties than silver nanoparticles and pluronic-coated silver nanoprisms. More importantly, the MICs for the Ag/SA against the *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were 0.51 and 0.26μg/mL respectively after 30 days. The MICs for the Ag/SA appeared an increase, while the MICs were still lower than MICs for nano-silver and pluronic-coated silver nanoprisms. It is important to note here that even if the polymer pluronic is completely safe for humans, the monomer is toxic. However, SA which is natural polysaccharide is nontoxic to humans. These results indicated that the Ag/SA composites could inhibit the *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 for a period of time.

The antibacterial properties of cotton fabrics dripped with the Ag/SA solutions were investigated using a modified Kirby-Bauer technique. Cotton fabrics were made into disks and placed on a bed of *S. aureus* ATCC 25923 in an agar plate. The antibacterial properties were measured by evaluating the zone of inhibition around the disk after incubation at 37□. Fig.4 and Table 2 appeared that the zones of inhibition for the B, C, D, E were approximately 1.8, 2.1, 3.4, 4.8mm respectively whereas there was no inhibition zone in A. Obviously, The zone of inhibition also increased, following the increase of nano-silver. These results revealed that the antibacterial activity was due to the presence of nano-silver. In Fig.5 and Table 3, after cotton fabrics containing the Ag/SA composites were washed 10 times, the zone of inhibition decreased from 4.7 to 3.0mm. When the cotton fabrics

dripped with the Ag/SA composites solution were dipped into the saturated solution of calcium chloride, the G blocks in different alginate chains form ionic crosslinks through $Ca^{2+}$ divalent cations, resulting in a network on the surface of cotton fabrics.[25] Besides, Cotton fabrics have a large number of functional groups such as hydroxyl, carbonyl groups that can combine with the SA which contains hydroxyl, carboxyl via hydrogen bonds. These results revealed that the cotton fabrics containing the Ag/SA composites exhibited antibacterial properties and owned washing resistance.

## 4. Conclusion

Highly stable, SA-capped silver nanoparticles with the size ranged from 20 to 30 nm were successfully synthesized through the method of in-situ reaction liquid phase reduction. In the Ag/SA, the nano-silver was evenly dispersed in the SA with no aggregation and the thermal stability of Ag/SA composites was improved. According to the antibacterial experiments, MICs for Ag/SA composites against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were 0.25 and 0.13μg/mL severally, even after 30days, the Ag/SA composites still presented antibacterial properties. The cotton fabrics containing Ag/SA composites showed antimicrobial activity and washing resistance. In the preparation of Ag/SA composites, the reaction conditions were moderate, and the reagents were non-toxic and had good biocompatibility. There is no doubt that these novel antibacterial materials are expected to be used in medical fields and biological medicines.

## Acknowledgements

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## Caption list

**Figure 1**. UV-vis absorption spectrum (a), TEM image (b) and XRD pattern (c) of silver nanoparticles (reaction between 1mL of 0.04M $AgNO_3$ and 20mL of 10mg/mL SA).

**Figure 2**. TG profiles of SA and Ag/SA (nano-silver content at about 1.57wt% ).

**Figuer 3**. Representative microplate wells for antimicrobial actions (at 24 h). A1 and D1: 10mg SA/mL, B1 and C1: (10mg SA+0.16mg nanosilver) /mL, Column 1 to 12: serial doubling dilutions.

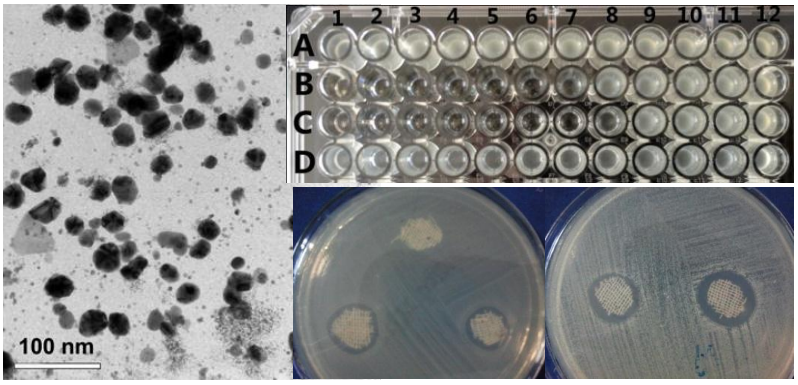
**Figure 4**. Diagram of inhibition zone experiment against *S. aureus* ATCC 25923 using cotton fabrics dripped with different solutions. A: 10mg/mL SA, B: (10mg SA+0.16mg nanosilver) /mL, C: (10mg SA+0.31mg nanosilver) /mL, D: (10mg SA+0.48mg nanosilver) /mL, E: (10mg SA+0.57mg nanosilver) /mL.

**Figure 5**. Diagram of inhibition zone experiment against *S. aureus* ATCC 25923 using cotton fabrics dripped by Ag/SA composites solution (0.57mg/mL nanosilver) after a certain number of washing. A: 0 time, B: 2 times, C: 5 times, D: 10 times.

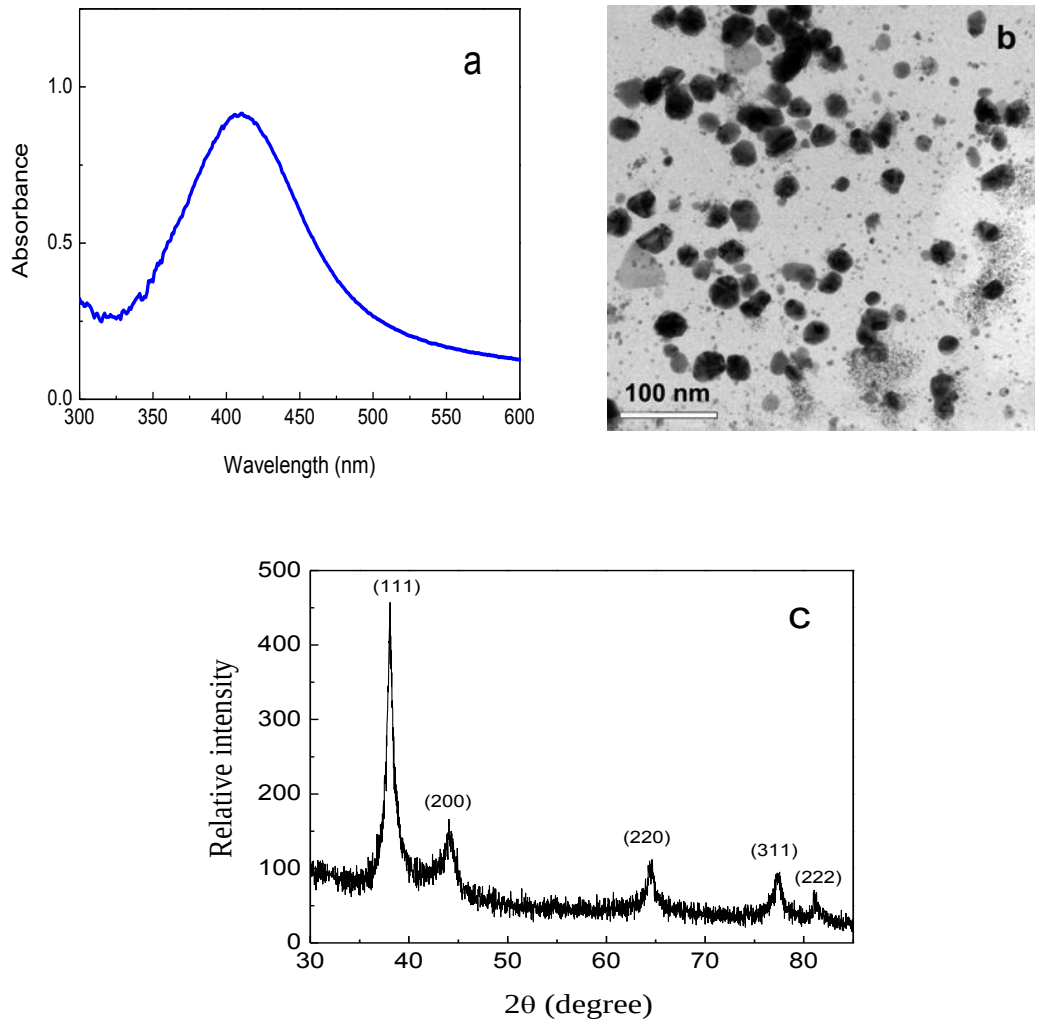
**Table 1**. Antibacterial properties of the Ag/SA against *S. aureus* ATCC 25923and *E. coli* ATCC 25922.

**Table 2**. Antimicrobial properties of cotton fabrics containing Ag/SA against *S. aureus* ATCC 25923.

**Table 3**. Antimicrobial activity of cotton fabrics containing Ag/SA composites solutions (0.57mg/mL nanosilver) against *S. aureus* ATCC 25923 after washed a certain number of times.




Nano-silver/sodium alginate composites that showed antibacterial activity towards *Staphylococcus aureus* and *Escherichia coli* were synthesized by an effective strategy.

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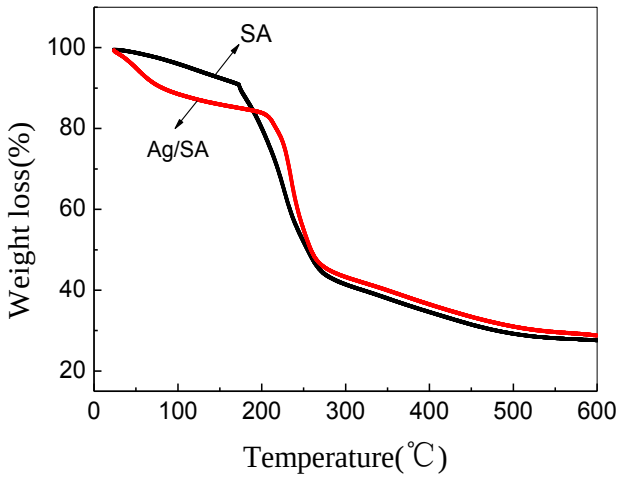


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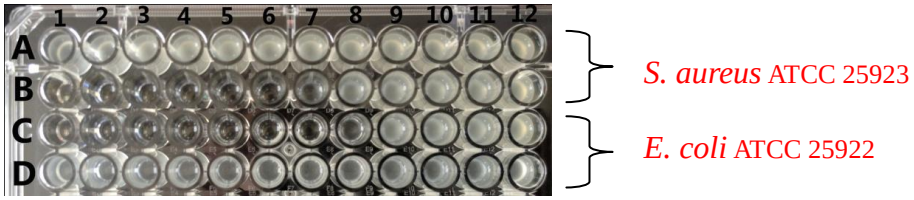


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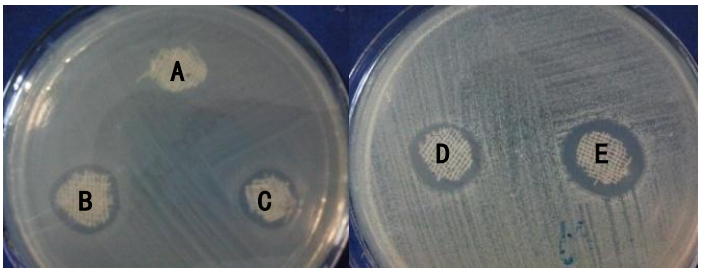


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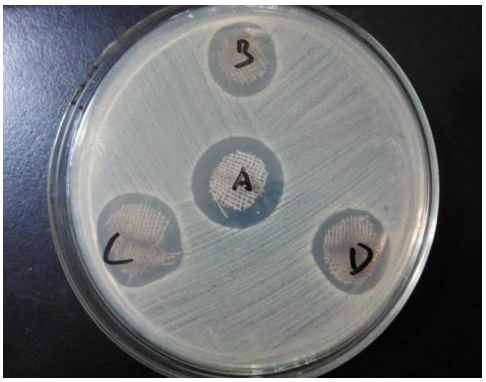


Figure 5. Diagram of inhibition zone experiment against *S. aureus* ATCC 25923 using cotton fabrics dripped by Ag/SA composites solution (0.57mg/mL nanosilver) after a certain number of washing. A: 0 time, B: 2 times, C: 5 times, D: 10 times.

Table 1. Antibacterial properties of the Ag/SA against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

| Experimental systems | 1day | | 30 days | |
|---|---|---|---|---|
| | NPW* | MIC(μg/mL) | NPW* | MIC(μg/mL) |
| A (SA, *S. aureus* ATCC 25923) | 0 | - | 0 | - |
| B (SA+nanosilver, *S. aureus* ATCC 25923) | 6 | 0.25 | 5 | 0.51 |
| C (SA+nanosilver, *E. coli* ATCC 25922) | 7 | 0.13 | 6 | 0.26 |
| D (SA, *E. coli* ATCC 25922) | 0 | - | 0 | - |

* NPW: number of pellucid well

Table 2. Antimicrobial properties of cotton fabrics containing Ag/SA against *S. aureus* ATCC 25923.

| Samples | Zone of inhibition(mm) |
|---|---|
| A (SA) | 0 |
| B (SA+0.16mg/mL nanosilver) | 1.8 |
| C (SA+0.31mg/mL nanosilver) | 2.1 |
| D (SA+0.48mg/mL nanosilver) | 3.4 |
| E (SA+0.57mg/mL nanosilver) | 4.8 |

Table 3. Antimicrobial activity of cotton fabrics containing Ag/SA composites solutions (0.57mg/mL nanosilver) against *S. aureus* ATCC 25923 after washed a certain number of times

| Washing times | Zone of inhibition(mm) |
|---|---|
| 0 | 4.7 |
| 2 | 4.2 |
| 5 | 3.6 |
| 10 | 3.0 |