



Silver-reinforced cellulose hybrids with enhanced antibacterial activity: Synthesis, characterization, and mechanism

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Article

Silver-reinforced cellulose hybrids with enhanced antibacterial activity: Synthesis, characterization, and mechanism

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In this article, silver particles filled cellulose hybrids were successfully synthesized using microcrystalline cellulose solution, AgNO₃, and AlCl₃·6H₂O by a hydrothermal method. Experimental results indicated the existence of silver nanoparticles in the cellulose matrix. Silver nanoparticles were well dispersed on the surface of cellulose and penetrated into the cellulose network. The microcrystalline cellulose solution played an important role in the synthesis of silver crystals. The reducing mechanism from Ag⁺ to silver was proposed in detail. Antibacterial experimental results displayed that the as-prepared hybrids exhibited excellent antimicrobial activities against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). This green strategy for the synthesis of cellulose-silver hybrids may be useful for the extensive applications of inorganic-polymer hybrids.

1. Introduction

Antimicrobial hybrids have been receiving more and more attention due to its widely applications in the fields such as water treatment, wound dressing, food packaging, and textiles.^{1,2} It is well known that silver has potential applications as antimicrobial materials in biomedical filed, antibacterial filters, and food packaging, thanks to its excellent antibacterial activity and relatively low toxicity to humans.³⁻⁶ Silver was reported to have strong antimicrobial activity against nearly 650 types of bacteria.⁵ Cellulose is one of the most abundant biomass in the nature, which is widely used in bio-ethanol, value-added materials, as well as bio-based chemicals. For example, cellulose was selectively converted into ethylene glycol and ethylene glycol monoether on NbOPO₄ supported Ru-based catalysts with methanol as the solvent.⁷ Cellulose was converted into n-Hexane over the Ir-ReO_x/SiO₂ catalyst combined with HZSM-5.⁸ Liu *et al.* reported the preparation of cellulose/polysulfonamide composite membrane as a high performance lithium-ion battery separator.⁹ Guo *et al.* successfully prepared the flexible, highly transparent, cross-linked cellulose thin film with high mechanical strength and low coefficient of thermal expansion.¹⁰ In the literatures, hybrids from cellulose and silver had also been extensively explored.¹¹⁻¹⁴ It was reported the synthesis of cellulose-silver hybrids with

antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* by using two distinct methodologies and two cellulose substrates of vegetable and bacterial cellulose.¹² Magnetic bacterial cellulose-silver hybrids with high antimicrobial activity were carried out using polydopamine as reducing reagent and stabilizer.¹⁴ Maneerung *et al.* demonstrated that silver nanoparticles were impregnated into bacterial cellulose with a strong antibacterial activity for antimicrobial wound dressing.¹⁵ Moreover, it was found that antimicrobial bacterial cellulose-silver/AgCl hybrids were achieved by using bacteria as a versatile biofactory.¹⁶ Among these literatures, choosing appropriate reducing agents for the synthesis of hybrids from cellulose and silver is of great importance for broadening and improving their industrial applications.

The fabrication of metal nanoparticles using naturally produced bio-derived reducing agents is a promising strategy in recent years. Many efforts have been made on the synthesis of metal nanoparticles by bio-derived reducing agents.¹⁷⁻²² Au, Ag, and Au-Ag nanoparticles with the quantum size domain were synthesized in water by using glucose as the reducing agent and starch as the protecting agent.¹⁷ In spite of that, silver and palladium nanoparticles were also obtained at room temperature using coffee and tea extract.¹⁸ It was noted that selenium nanoparticles with 10-20 nm in diameter had been prepared using nanocrystal cellulose as the reducing and structure-directing agent under hydrothermal conditions.¹⁹ Then, nanocrystalline cellulose from cotton was also used as the reducing agent for the synthesis of platinum nanoparticles by reduction of H₂PtCl₆.²⁰ After that, spherical and cubic Pt nanoparticles with high catalytic activities were synthesized using wood nanomaterials in aqueous phase.²¹ Pt nanoparticles had also been carried out at relatively low temperature in aqueous solution from hexachloroplatinic acid using nanocrystal cellulose from cotton as reducing agent.²² Zhang's group had successfully synthesized Pd and Ag nanoparticles using cellulose nanocrystals or cellulose hydrogel as reductants for antibacterial and catalytic applications.²³⁻²⁵ As described above, cellulose displayed reducing ability due to its low molecular weight and short carbochain.²⁶⁻²⁸

Herein, hybrids from cellulose and silver were carried out using microcrystalline cellulose solution, AgNO₃, and AlCl₃·6H₂O by the hydrothermal method. In comparison with others methods, the hydrothermal method has the advantages of mild reaction conditions, enhancing reactivity, energy-saving, and preparing the products with high crystalline, high purity, narrow size distribution, and good dispersion. The microcrystalline

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cellulose was dissolved in NaOH/urea aqueous solution. The as-prepared products exhibited good antibacterial activities against both *E. coli* and *S. aureus*. It is believed that this green strategy is hopeful to be expanded in the synthesis of other metal-contained hybrids, which may open a new window for the extensive high value-added applications of biomass.

2. Experimental section

2.1. Materials

All chemicals used in our experiments were purchased and used as received without further purification. All experiments were operated under an ambient atmosphere. Microcrystalline cellulose (MCC, molecular weight of 34, 843–38, 894, degree of polymerization (DP, DP = 215–240)) of a commercial reagent was purchased from Sinopharm Group Chemical Reagent Co., Ltd., Shanghai, China). $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and AgNO_3 were purchased from Guangzhou Jinhua Chemical Reagent Co., Ltd. Urea was purchased from Guangzhou XiLong Chemical Co., Ltd. NaOH was purchased from Beijing Chemical Works.

2.2. Preparation of hybrids from microcrystalline cellulose and silver

The microcrystalline cellulose solution was obtained as previously described.²⁹ In a typical synthesis, 7.00 g of NaOH and 12.00 g of urea were added into 81 mL of distilled water under vigorous stirring to form NaOH-urea aqueous solution. Then 3.24 g microcrystalline cellulose (MCC) was added immediately into the above solution under vigorous stirring at room temperature, and then the suspension solution was cooled down to $-12\text{ }^\circ\text{C}$ for 12 h. The obtained cellulose solution was used for the fabrication of cellulose-silver hybrids. The molecular weight of MCC before and after dissolved in NaOH/urea was measured by copper ethylenediamine viscosity method. Firstly, MCC was dissolved in upriethy lenediamine solution to form MCC solution, then the MCC solution was added into ubbelohde viscometer to measure its intrinsic viscosity $[\eta]$. The degree of polymerization (DP) of MCC can be obtained by the equation: $\text{DP}^{0.905} = 0.75 [\eta]$. After NaOH/urea treatment, the molecular weight of the cellulose was calculated from their degree of polymerization by multiplying with 162. The value of degree of polymerization of the cellulose is 150. Therefore, the molecular weight of regenerated cellulose is 24,300.

For the synthesis of cellulose-silver hybrids, 5 mL of AgNO_3 solution (0.20 mol L^{-1}) and 5 mL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution (0.20 mol L^{-1}) were added into the above obtained cellulose solution (5 mL). The mixture solution was transferred into a 25-mL Teflon-lined stainless steel autoclave. The autoclave was maintained at a certain temperature (from 120 to $180\text{ }^\circ\text{C}$) for a certain time (from 3–12 h). The product was separated from the solution by centrifugation, washed by water and ethanol several times and dried at $60\text{ }^\circ\text{C}$ for further characterization.

2.3. Antimicrobial activity studies

The antimicrobial activities of hybrids from cellulose and silver were investigated against *E. coli* as the model Gram-negative bacteria and *S. aureus* as the model Gram-positive bacteria by the disc diffusion method. In the inhibition zone experiment, nutrient agar was poured into disposable sterilized Petri dish and solidified. Then $100\text{ }\mu\text{L}$ of *E. coli* and $100\text{ }\mu\text{L}$ of *S. aureus* were streaked over the dish and spread uniform. After that,

circular pieces of the control and the test samples were gently placed on Petri dishes. This was done for both the bacterial strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923). The hybrids from cellulose and silver were cut into a disc shape with 1.4 cm diameter, sterilized by autoclaving at $120\text{ }^\circ\text{C}$ for 20 min, and placed on *E. coli*-cultured and *S. aureus*-cultured agar plates, which were then incubated at $37\text{ }^\circ\text{C}$ for 24 h. Finally, the inhibition zone was monitored.

2.4. Cell Cytotoxicity

The human gastric carcinoma cells (MGC_803) that were cultured in a RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillinstreptomycin at $37\text{ }^\circ\text{C}$ for 36 h, were used for cell viability test. Then, the cells were seeded in 96 well flatbottom microassay plates at a concentration of $1 \times 10^4\text{ cells mL}^{-1}$ and cultured for 24 h. The sterilized samples were added into wells at the concentration from 0.1, 1, 10 to $100\text{ }\mu\text{g mL}^{-1}$, and were cultured with cells for 36 h. The sample free tissue culture plate was used as a control. Cell viability was quantified by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and the data are representative as mean value of five parallel experiments.

2.5. Characterization

X-ray powder diffraction (XRD) patterns were obtained in 2θ range from 10° to 70° on a Rigaku D/Max 2200-PC diffractometer with Cu $K\alpha$ radiation ($\lambda = 0.15418\text{ nm}$) and graphite monochromator at ambient temperature. The morphologies of cellulose-based hybrids were examined by scanning electron microscopy (SEM, Hitachi 3400 N, accelerating voltage 15 kV), transmission electron microscopy (TEM, HT7700, accelerating voltage 120 kV), and high-resolution transmission electron microscopy (HRTEM, JEM-2100F, accelerating voltage 200 kV). All samples were Au coated prior to examination by SEM. Solid-state ^{13}C cross-polarization magic angle spinning (CP/MAS) NMR spectra were obtained on a Bruker AVIII 400 Mhz spectrometer. The X-ray photoelectron spectroscopy (XPS) analysis was performed on a ESCALAB 250Xi X-ray photoelectron spectroscope with a monochromatic Al $K\alpha$ radiation ($h\nu = 1486.6\text{ eV}$) and the banding energies were normalized to C1 s peak at 284.8 eV (Thermo Scientific Co., England). The concentration of Ag^+ ions in solution was analyzed by inductively coupled plasma (ICP, OPTIMA8X00, American).

3. Results and discussion

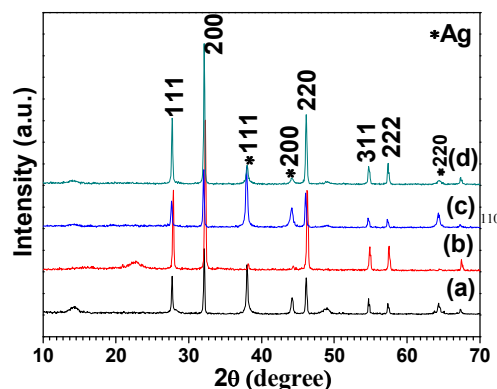


Fig. 1. XRD patterns of the hybrids synthesized by hydrothermal method for 12 h: (a) NaOH/urea solution without dissolution of MCC; (b) 0.324 g MCC; (c) 2 mL cellulose solution (dissolution the MCC in NaOH/urea solution); (d) 1 mL cellulose solution (dissolution the MCC in NaOH/urea solution).

The X-ray diffraction (XRD) was widely used in examining the crystallographic nature to confirm the formation of nanoparticles. **Fig. 1a** showed XRD pattern of the sample synthesized using AgNO_3 , $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and NaOH/urea solution (without dissolution the MCC). One can observe that the sample consisted of mixed phases of well-crystallized AgCl with a cubic structure (JCPDS 31-1238) and silver (Marked with * in the **Fig. 1(a)**). **Fig. 1b** showed XRD pattern of sample synthesized using AgNO_3 , $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and MCC (without dissolution in NaOH/urea solvent), while other conditions were kept the same. The well-crystallized AgCl crystals were observed as a major phase and silver crystals were still existed as a minor phase. **Fig. 2** shows the XRD patterns of the typical samples prepared using AgNO_3 , $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and MCC solution (dissolution the MCC in NaOH/urea solution) by the hydrothermal method at 160 °C for 3, 6, 9, and 12 h, respectively. One can see that all of the samples exhibited similar diffraction peaks at $2\theta = 38.0^\circ$, 44.2° , and 64.4° , which could be assigned to the (111), (200), and (220) planes of the crystallized silver with a cubic structure (JCPDS 04-783). No obvious peaks of cellulose were observed, which might due to the strong peaks intensities of silver crystals. The Ag contents in the hybrids for the samples synthesized for 3, 6, 9, and 12 h were 43.8%, 47.1%, 49.8%, and 36.9%, respectively. There is a slight difference on the Ag contents in the hybrids with increase heating time. Moreover, no peaks from AgCl were obtained, which implied the complete reduction of Ag^+ to metal silver. In the experimental system, no other agents were added into this experimental system. These results demonstrated that microcrystalline cellulose solution is considered to play a vital role in the synthetic process of silver crystals in the hybrids. In the previous literatures, metal crystals could be obtained using nanocrystal cellulose as reducing reagent due to its low molecular weight and short carbochain.¹⁹⁻²² MCC possesses plenty of hydroxyl groups, which has weak reducing ability.³⁰

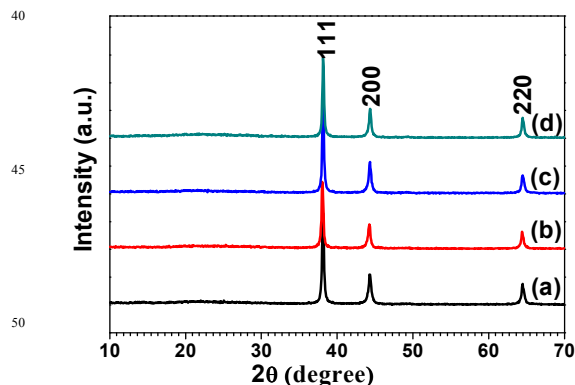
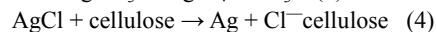
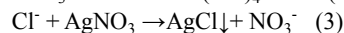
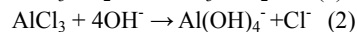


Fig. 2. XRD patterns of the hybrids synthesized by hydrothermal method at 160 °C for different times: (a) 3 h; (b) 6 h; (c) 9 h; (d) 12 h.

In the synthetic process, the possible chemical reactions for the precipitation of AgCl crystals can be described by the Equations

(1)-(4).



At last, Ag crystals were obtained by reduction of Ag^+ by the hydrothermal method.

In order to explore the mechanism in reducing Ag^+ ions, further experiments were carried out in our study. Based on the mentioned above results, MCC solution (dissolution the MCC in NaOH/urea solution) played an important role in the synthesis of silver crystals. As shown in **Fig. 1a**, no pure phase of silver crystals were obtained in the hybrids without MCC. As shown in **Fig. 1b**, no pure phase of silver crystals were obtained in the hybrids with MCC. These results indicated that it did not obtain silver crystals using NaOH/urea solution and MCC. However, it obtained silver crystals using MCC solution (dissolution the MCC in NaOH/urea solution) (**Fig. 2**). Obviously, it was due to the interactions between MCC and NaOH/urea solution.

As is known, cellulose is composed of β -(1 \rightarrow 4)-linked D-glucopyranosyl units with three hydroxyl groups, which can form complex inter- and intra-molecular hydrogen bonds.³¹ Thus, cellulose usually possesses high crystallinity and cannot be degraded easily; it only exhibits weak reduction capacity. However, when treated with NaOH/urea solution, most of linkages such as hydrogen bonds in the cellulose were destroyed, the long glucose chains were broken into shorten ones, and large amounts of reducing ends occurred. The untreated MCC has molecular weight of 34,843-38,894 and degree of polymerization of 215-240. After NaOH/urea treatment, the cellulose has molecular weight of 24,300 and degree of polymerization of 150. Therefore, the degree of polymerization of treated cellulose was dramatically decreased, implying that the increasing reducing ends were obtained, compared with that of untreated MCC. Obviously, it is believed that the treated MCC usually exhibits relatively weak reduction capability for Ag^+ ions. It is reported that the synthesis of gold crystals in water revealed the reduction process using glucose as the reducing agent in the previous literature.¹⁷ In the formation process of metal gold crystals, the base facilitates the opening of the glucose ring by the abstraction of the α -proton of the sugar ring oxygen and the metal ions oxidize glucose to gluconic acid. It seems that the formation process of metal silver is similar to that of gold crystals.

Investigation of the influence of MCC solution volume (dissolution the MCC in NaOH/urea solution) on the synthesis of silver crystals was also conducted. From **Fig. 1d**, when 1 mL of the cellulose solution was used, AgCl crystals were observed as the major phase while silver existed as the minor phase. Increasing the cellulose solution volume to 2 mL, silver crystals were obtained as the major phase while AgCl existed as the minor phase (**Fig. 1c**). The diffraction peaks of AgCl disappeared, only silver diffraction peaks existed, and the complete reduction of Ag^+ to metal silver was observed with further increasing cellulose solution volume to 5 mL (**Fig. 2d**). These results suggested that the silver peaks intensities increased obviously with increasing cellulose solution volumes, which was in good agreement with the above mentioned reduction mechanism.

All of the above results indicated the successful fabrication of metal silver using MCC solution (with dissolution MCC in NaOH/urea solution) under mild hydrothermal treatment. Although the mechanism describing the formation of metal silver is complex, it is believed that the presence of MCC solution is necessary. The schematic illustration for the formation of cellulose-silver hybrids was showed in **Fig. 3**. It was reported that the formation of metal crystals using cellulose as matrix involved the nucleation, growth, and aggregation procedures.^{32,33} Taking MCC for instance, firstly, MCC was dissolved in NaOH/urea solution and formed cellulose solution. When cellulose solution was mixed with AgNO₃ solution and AlCl₃·6H₂O solution, Ag⁺ reacted with Cl⁻ to form AgCl crystals and some Ag⁺ diffused into the matrix and anchored on the surface of cellulose probably via electrostatic interactions by the electron-rich oxygen atoms of hydroxyl and ether groups of cellulose. After that, the absorbed silver ions inside the cellulose matrix were reduced to the metallic silver crystals under the hydrothermal reaction condition. Of course, the intrinsic mechanism at the molecular level is also needed to be further studied in detail in the near future.

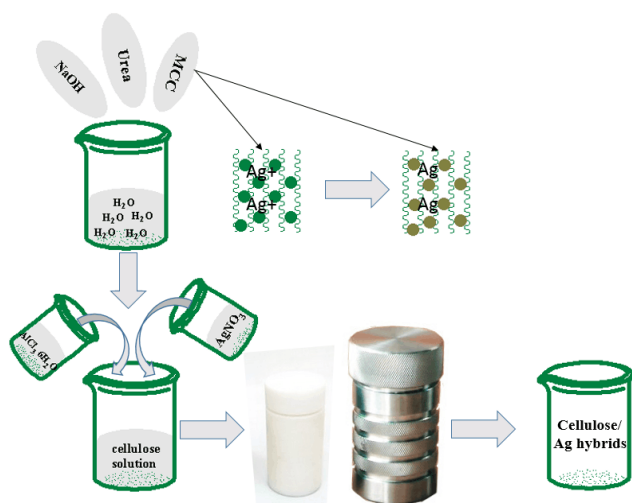


Fig. 3. Schematic representation of the formation mechanism of cellulose-silver hybrids.

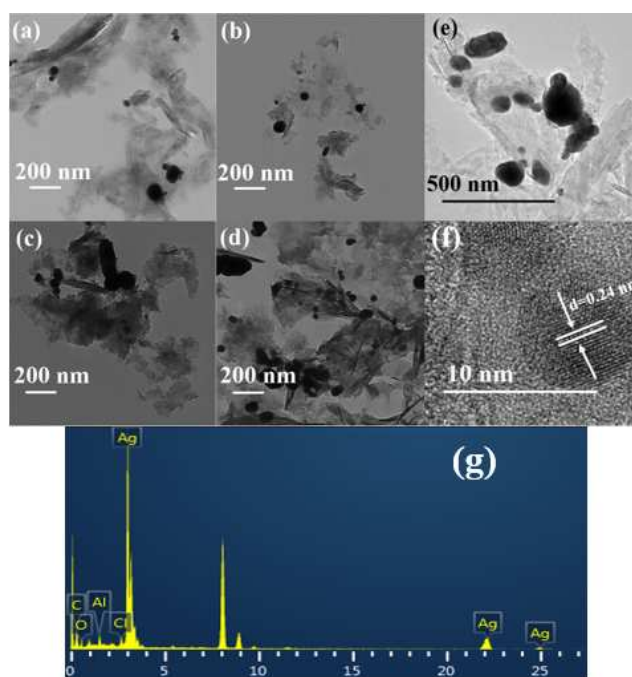


Fig. 4. TEM images of the hybrids synthesized by hydrothermal method at 160 °C for different times: (a) 3 h, (b) 6 h, (c) 9 h, (d) 12 h, respectively; (e) TEM image, (f) HR-TEM image, (g) corresponding EDX spectrum of Ag in cellulose-silver hybrids synthesized at 160 °C for 12 h.

The morphologies and microstructures of the cellulose-silver hybrids were further examined by scanning electron microscopy (SEM). **Fig. 4** shows the SEM images of cellulose-silver hybrids synthesized by hydrothermal method at 160 °C for 3, 6, 9, and 12 h, respectively. From **Fig. 4a and b**, one can observe that cellulose was decorated with silver particles, but it was difficult to distinguish the structures and morphologies between cellulose and Ag particles. Some clusters of the silver particles were evident. Therefore, it was very difficult to make an accurate estimation of the average particle size. Most of the clusters disappeared and some individual silver particles were detected with increasing heating time (**Fig. 4c and d**), indicating that long reaction time favored for the well dispersion of silver particles in the cellulose matrix. Long reaction time favored for the precipitate of cellulose, which provided a matrix for the synthesis of Ag. Moreover, Long reaction time also favored for the growth of silver particles, which were well dispersed in the cellulose matrix. Of course, the intrinsic mechanism needs to be further explored in the near future.

The structures of cellulose-silver hybrid synthesized by hydrothermal method at 160 °C for 12 h were further examined with TEM and HR-TEM (**Fig. 4e and f**). One can observe the Ag particles with irregular shapes in the cellulose matrix (**Fig. 4e**). HR-TEM image presented the lattice spacing determined to be 0.24 nm, which was indexed to the d spacing of the Ag (111) plane (**Fig. 4f**). HR-TEM results further confirmed that the surface of cellulose was decorated by the Ag particles. The corresponding EDX spectrum of cellulose-silver hybrid was shown in **Fig. 4g**. All the peaks were attributed to Ag, C, O, Al,

and Cl, which were indicative of the Ag, MCC and the residue of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ present in the system. **Fig. S2a** shows the histogram of Ag particle size distribution of the same sample. The diameters of Ag particles were in the range of 20-250 nm. The average diameter of Ag nanoparticles was 85.2 nm.

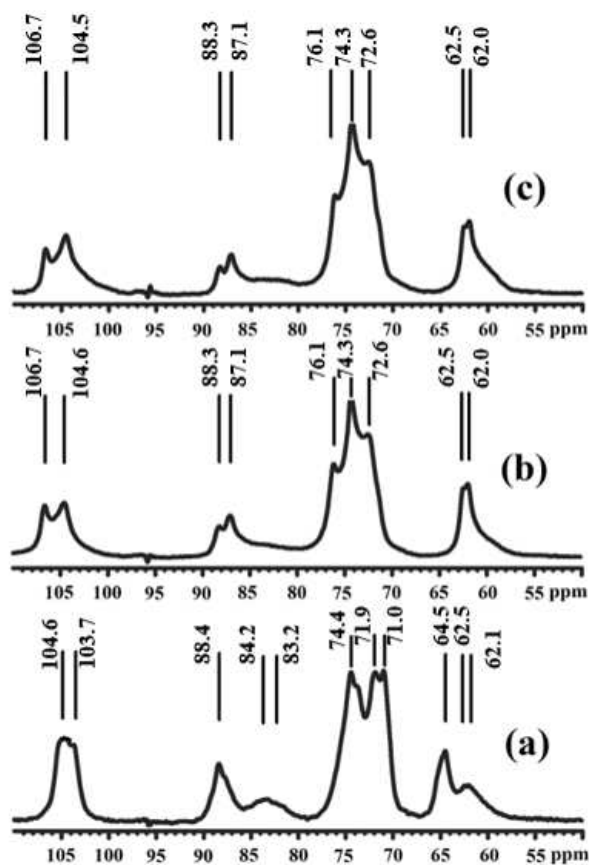


Fig. 5. CP/MAS ^{13}C -NMR spectra of: (a) MCC; (b) the regenerated MCC from NaOH/urea solvent; (c) cellulose-silver hybrids synthesized at 160°C for 6 h.

Fig. 5a shows the cross polarization magic angle spinning (CP/MAS) ^{13}C solid-state NMR spectrum of the pure MCC. The signals at (62.1+62.5+64.5), (71.0+71.9+74.4), (83.2+84.2+88.4), and (103.7+104.6) ppm are attributed to C-6, C2-C3-C5, C-4, and C-1, respectively.³⁴ In addition, the small peaks at about 62.1, 62.5, 83.2, and 84.2 ppm are the contribution of the disordered regions for C6 and C4 carbons; the peaks at about 64.5 and 88.4 ppm stand for the ordered regions for C6 and C4 carbons, respectively.³⁵ For comparison, the CP/MAS ^{13}C solid-state NMR spectra of the MCC treated with NaOH/urea solution and cellulose-silver hybrids synthesized by hydrothermal method using AgNO_3 , $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, and cellulose solution at 160°C for 6 h are also shown in **Fig. 5b and c**, respectively. In the two spectra, similar noticeable signals are observed in the region between 60 and 110 ppm, which are mostly attributed to the different carbons of cellulose. The signals at (62.0+62.5), (87.1+88.2), and (104.5+106.7) ppm are assigned to C-1, C-4, and C-6, respectively. One can observe that the signal at 64.5 ppm dramatically disappeared in **Fig. 5b and c**. It is shown that the

30 peaks at around 104.6 and 88.4 ppm are split and the peak intensities decreased, compared with **Fig. 5a**. These results indicate that the NaOH/urea pretreatment has an influence on the crystallinity of cellulose. At the same time, it is worth noting that a slight difference is detected in the peaks at around 104 ppm. The peaks are located at around 104.6, 106.7, and 106.7 ppm in **Fig. 5**. The peak moved from 104.6 to 106.7 ppm by the NaOH/urea pretreatment, demonstrating that the hydrogen bonds increased, glycosidic bonds of cellulose broke, and new reducing ends produced. This experiment result further supported the previous suggested reduction mechanism.

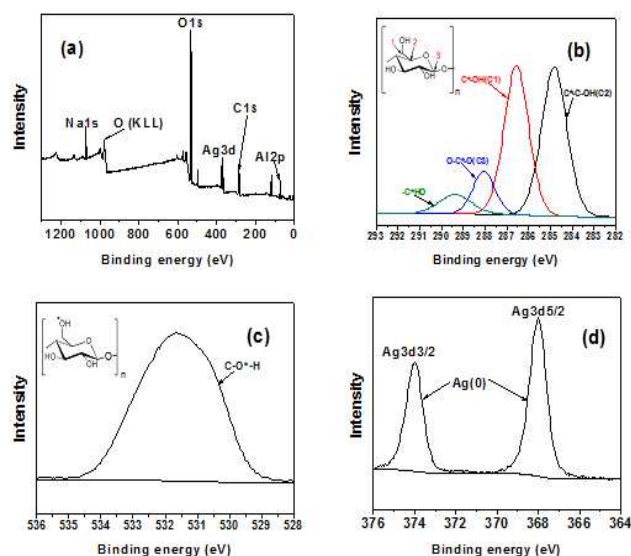


Fig. 6. Scanning XPS spectrum of (a) cellulose/Ag/AgCl hybrids, and (b-d) high-resolution XPS spectra of (b) C 1s, (c) O 1s, and (d) Ag 3d.

The XPS spectra were carried out to further determine the chemical compositions and the valence states of the prepared species. A typical survey-scan XPS spectrum was shown in **Fig. 6a**. One can observe clearly Na, C, O, Ag, and Al elements. The corresponding high-resolution XPS spectra of C 1s, O 1s, and Ag 3d orbits were also obtained (**Fig. 6b-d**). One can see three apparent peaks in the C 1s curve, which represent three different chemical environments. The binding energies at 284.8, 286.5, and 288.0 eV can be assigned to the C*-C-OH, C*-OH, and O-C*-O bonds, individually,³⁶ manifesting that the carbon atoms derive from glycosyl units in the cellulose. There exist one peak at 531.6 eV in the high-resolution O 1s XPS spectrum, which can be due to C-O*-H band. Two peaks are found in the high-resolution Ag 3d XPS spectrum. The peak at 368.0 eV can be attributed to the Ag 3d5/2 orbit, and the other peak at 373.9 eV can be assigned to Ag 3d3/2 orbit. Both the two peaks at 368.0 and 373.9 eV are attributed to Ag (0),³⁷ indicating that the sample is composed of a single phase of silver, which is in good accordance with the XRD result in **Fig. 1**. Based on the results of XPS analysis, silver crystals have been synthesized after the reaction, further confirming that Ag^+ is completely reduced to Ag (0) by cellulose solution.

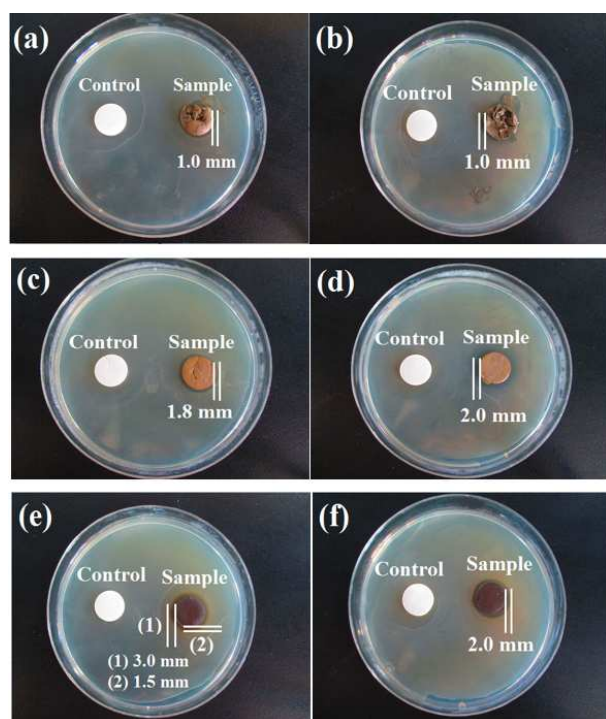


Fig. 7. Antimicrobial activities of cellulose/Ag/AgCl hybrids synthesized at 160 °C for 12 h: (a and b) cellulose solution; (c and d) NaOH/urea solution; (e and f) 0.324 g MCC.

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The antibacterial experiments against *E. coli* and *S. aureus* were also carried out to give a further exploring in the antibacterial activities of the cellulose-silver hybrids. As shown in **Fig. 7**, pure MCC was used as control. No inhibition zone was observed for MCC, indicating that pure cellulose alone had no antibacterial properties. It is apparent inhibition zone occurred for the cellulose-silver hybrids. From **Fig. 7a and b**, one can see that the inhibition zones of the cellulose-silver hybrids synthesized using cellulose solution (dissolution the MCC in NaOH/urea solution) for *E. coli* and *S. aureus* were the same as 1.0 mm. **Fig. 7c and d** showed that the inhibition zones of the cellulose/Ag/AgCl hybrids synthesized using NaOH/urea solution (without MCC) for *E. coli* and *S. aureus* were 1.8 mm and 2.0 mm, respectively. The inhibition zones of the cellulose/Ag/AgCl hybrids synthesized using MCC (without dissolution in NaOH/urea solution) for *E. coli* and *S. aureus* were 1.5 mm/3.0 mm (inner/outer) and 2.0 mm, respectively (**Fig. 7e and f**). The results showed that the as-prepared cellulose-based hybrids had good antibacterial activities against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). According to the results of antibacterial experiments, one can also conclude that the antimicrobial activity against *E. coli* was relatively lower than that against *S. aureus*, which might be assigned to the difference in cell walls between Gram-negative and Gram-positive bacteria.³⁸ It is of interest to observe two inhibition zones of cellulose/Ag/AgCl hybrids for *E. coli*, and this can be due to the releasing behavior of silver and AgCl crystals in the hybrids.³⁹ It was thought that the silver and AgCl crystals located in the surface region of the cellulose matrix were ready to release when incubated with bacteria, while the silver and AgCl crystals

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impregnated in the inner part of the composite were hard to diffuse out due to the special structure of cellulose, thus the silver and AgCl crystals went into a sustained release way. This implied that a prolonged antibacterial effect was promising to be achieved by cellulose/Ag/AgCl hybrids to increase the inhibition zone to 3.0 mm. It is well known that the size and size distribution of silver particles have significant influence on their antibacterial properties. The size distribution of the as-prepared hybrids was shown in **Fig. S2**. The mean size of corresponding particles were 85.2 nm, 5.3 μm , and 1.7 μm , respectively. One can observe that the cellulose/Ag/AgCl hybrids with relatively small size displayed high antibacterial property. What's more, the Ag^+ releasing concentrations for different times were investigated by ICP. The Ag^+ concentrations were 0.091 ppm, 0.073 ppm, and 0.080 ppm in solutions (using H_2O as solvent) for 4, 12, and 24 h, respectively. These results clearly demonstrated that the antibacterial activity of hybrids was due to the existence of silver crystals. Of course, the intrinsic mechanism of effects on the antibacterial properties of the as-prepared hybrids needs to be further investigated in the near future.

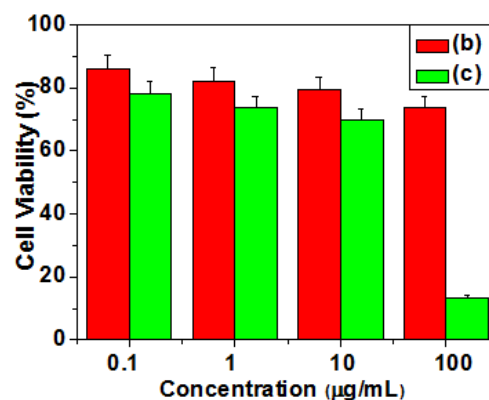
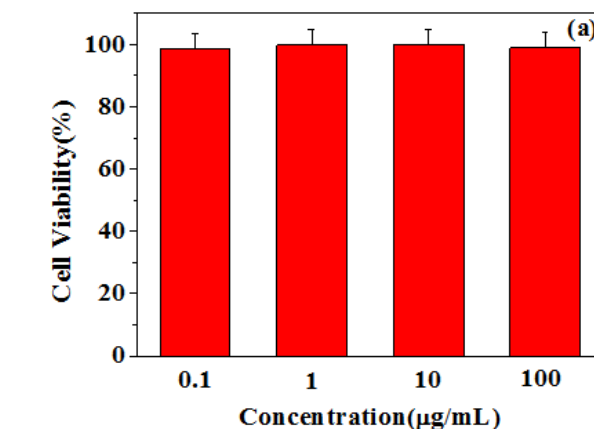


Fig. 8. Viabilities of normal human fibroblasts incubated with the hybrids: (a) initial microcrystalline cellulose (MCC), (b) cellulose/Ag hybrids, and (c) cellulose/Ag/AgCl hybrids. They were determined by survival cells per well relative to that of untreated cells. The error bars stand for standard deviations.

Cell cytotoxicities of the MCC, cellulose/Ag hybrids, and cellulose/Ag/AgCl hybrids were also investigated, as shown in

Fig. 8. As the control sample, the initial MCC cell cytotoxicity has been studied. When the MCC concentrations were 0.1, 1, 10, and 100 $\mu\text{g mL}^{-1}$, the cell viability values were 98.5%, 99.8%, 100% and 99.2%, respectively (**Fig. 8a**). The result indicated that initial MCC had no cell cytotoxicity. When the cellulose/Ag hybrids concentrations were 0.1, 1, 10, and 100 $\mu\text{g mL}^{-1}$, the cell viability values were 86.0%, 82.1%, 79.3% and 73.6%, respectively (**Fig. 8b**). The cell viability values decreased with increasing cellulose/Ag hybrids concentrations. However, as for the cellulose/Ag/AgCl hybrids, the cell viability values were 78.1%, 73.6%, 69.7%, and 13.1% with the cellulose/Ag/AgCl hybrids concentrations of 0.1, 1, 10, and 100 $\mu\text{g mL}^{-1}$ (**Fig. 8c**). These results indicated that the cell viability values dramatically decreased with increasing cellulose/Ag/AgCl hybrids concentrations. Especially, when the cellulose/Ag/AgCl hybrids concentration was 100 $\mu\text{g mL}^{-1}$, the cell viability value was only 13.1%. Low cell viability value indicated that cellulose/Ag/AgCl hybrids with high concentration had a strong cytotoxicity and bad biocompatibility. Furthermore, the cell viability values of cellulose/Ag hybrids were more than those of cellulose/Ag/AgCl hybrids, implying that cellulose/Ag hybrids have low cell cytotoxicity. These results were in accord with the previous results of antibacterial activity, in which cellulose/Ag/AgCl hybrids had high antibacterial effect, compared with cellulose/Ag hybrids.

4. Conclusions

In summary, the hybrids from cellulose and silver with excellent antimicrobial properties have been successfully synthesized using MCC solution through the hydrothermal method. Experimental results implied the cellulose-silver hybrids with the silver crystals dispersed in the cellulose matrix. The possible mechanism in this article was proposed in detail. The cellulose-silver hybrids exhibited a strong antibacterial activity against both *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria). These hybrids might be promising antibacterial candidate for the applications in the biomedical field. This synthetic strategy reported here opens a new window to the high value-added applications of cellulose.

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