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Preparation of Durable Antibacterial Cellulose with AgCl Nanoparticles

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Abstract: In this study, a facile method was developed to coat AgCl nanoparticles (NPs) onto knitted cotton fabrics. The AgCl NPs were characterized by ultraviolet absorption spectrum, X-ray diffraction (XRD) and dynamic laser light scattering (DLS). The AgCl NPs were coated onto cotton fabrics through a pad-dry-cure process with the assistance of 1,2,3,4-butanetetracarboxylic acid (BTCA). Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), ICP-OES analysis and energy-dispersive X-ray spectroscopy (EDX) confirmed that AgCl NPs were successfully coated onto cotton fabrics. The prepared cotton samples exhibited excellent antimicrobial activity against both Gram-positive *S. aureus* and Gram-negative *K. pneumonia* bacteria. Rat skin fibroblast cytotoxicity testing demonstrated the treated cotton fabrics to be non-toxic. The washing durability evaluation showed that the antimicrobial function of cotton fabrics was durable to washing. In addition, the wrinkle resistance of the coated cotton fabrics was improved and there was no obvious change in whiteness.

Keywords: Cotton fabric, Antibacterial, AgCl NPs, BTCA, Durability

Introduction

Cross-infections caused by health-care related textiles including medical gowns, bed sheets, uniforms, aprons are getting great attentions of the public and researchers [1-5]. Although ultraviolet radiation, steam and alcohol are widely used to sterilize textiles in hospital, bacteria can re-contaminate the textiles and still remain high risk of infections. So incorporating antibacterial agents into textiles is a promising way to prevent the microbial infections. During the past two decades, quaternary ammonium salts [6-8], N-halamine [9-12], peroxide [13,14] and nanomaterials [15-27], have been widely studied to develop antimicrobial fabrics.

Nano-sized antibacterial agents have received special attention due to its broad antibacterial activity and durability to microorganisms. Among these nano-sized antibacterial agents, silver nanoparticles (NPs) are widely used. However, the shortcoming of textile yellowing or browning caused by silver NPs limits their applications [15-17]. To solve this problem, many researchers and engineers showed their interests in light-colored silver salts, such as silver chloride. For example, Simončič *et al.* successfully embedded AgCl nanoparticles onto cotton, wool, PET, and PA fibers [23-25], and the modified fibers showed excellent antibacterial activity with little negative effect on whiteness. Khatri *et al.* coated a chitosan/AgCl-TiO_2 colloid system onto cotton fabrics to obtain antibacterial activity and improve wrinkle resistance [26]. Additionally, some commercially available AgCl NPs antibacterial agents were also developed, which can be bonded onto fibers with proper binders [27,28].

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In this study, durable Antibacterial cotton Fabrics coated with AgCl NPs were fabricated using a dip-pad-dry process. The AgCl NPs were characterized by UV spectra, XRD and DLS. The AgCl-coated cotton fabrics were characterized by FTIR, SEM, EDS and ICP-OES. *S. aureus* (ATCC 6538) and *K. pneumonia* (ATCC 4352) were used to evaluate the antibacterial efficacy of the treated cotton fabrics. The biocompatibility of the AgCl NPs modified cotton fabrics was assessed by a cell viability test. The mechanical property, wrinkle recovery angle, whiteness and specific surface area of the prepared cotton fabrics were also examined.

Experimental

Materials

Knitted cotton fabrics were purchased from Yuming Textile Co., Ltd., Shanghai, China. $AgNO_3$ and NaCl were supplied by Sinopharm Chemical Reagent Co., Ltd., Shanghai. BTCA was provided by Changzhou Chemical Industry Research Institute Co., Ltd., Changzhou, China. All of the chemicals were used directly without further purification. Commercially available Watson distilled water was applied in the experiment.

Preparation AgCl Colloid

The AgCl NPs colloid was prepared through the reaction between diluted silver nitrate and equimolar quantity of sodium chloride. Briefly, $AgNO_3$ (0.4 mmol) and BTCA (50 mmol) were added into distilled water (100 m*l*). After continuous stirring for 5 min, the mixture solution was slowly poured into 100 m*l* aqueous solution of NaCl (0.4 mmol) with vigorous stirring under ambient temperature to get a milky colloid.

Coating AgCl NPs onto Cotton Fabrics

After dipping into AgCl colloid for 5 min under constant stirring, the cotton fabrics were taken out and padded through a laboratory padder to get 80 % wet pick-up. Then the fabrics were dried at 100 °C for 3 min, followed by curing at 170 °C for 3 min. Finally, the cotton samples were washed thoroughly with excess water to remove any loosely bonded chemicals.

Characterization of AgCl NPs and AgCl NPs Coated Cotton Samples

UV-vis absorption spectra of AgCl NPs were examined by Lambda 900 UV-vis spectrophotometer (Perkin Elmer, USA) with wavelength from 200 to 800 nm. The particle size of AgCl NPs was detected by a Nano-ZS90 particle size measurement system (MALVERN, UK). The X-ray diffraction (XRD) patterns of AgCl NPs were recorded by a Bruker Instrument model D8. Fourier transform infrared (FTIR) spectra of samples were obtained with a Nicolet NEXUS 470 spectrometer (Nicolet Instrument Corporation, Madison, WI). The surface morphology of cotton fabrics was studied using a SU-1510 scanning electron microscope (Hitachi, Japan). The chemical composition of fiber surface was illustrated by energy dispersive spectroscopy (EDX, IE 3000, UK). The silver content of the AgCl NPs coated cotton fabrics was detected by ICP-OES (Perkin Elmer, USA). Before the ICP-OES measurements, the cotton fabrics were digested in nitric acid and hydrogen peroxide under microwave.

Antibacterial Efficacy Evaluation

The antibacterial efficacy of AgCl NPs coated cotton fabrics were evaluated according to ISO 20743-2013 (Textiles-Determination of antibacterial activity of antibacterial finished products: Absorption method). Untreated cotton samples acted as the control in the testing. All of the samples were autoclaved at 121 °C for 20 minutes for sterilization before the inoculation. *S. aureus* (ATCC 6538) and *K. pneumonia* (ATCC 4352) were chosen as the models for Gram-positive bacteria and Gram-negative bacteria, respectively. The concentrations of inoculums are 1×10^5-3×10^5 (CFU/m*l*). 0.2 m*l* of bacterial suspensions was inoculated onto 0.4 g cotton samples in vials. After inoculation, SCDLP broth (20 m*l*) was added immediately and the capes were tightened. The mixture was shaken for 5 s× 5 cycles using a vortex. The samples were incubated at 37 °C for 20 h. After incubation, the vials were added with SCDLP (20 m*l*) and shaken again. After serial dilution, plate count method was employed for the statistic of the viable bacteria. Each test was triplicate and the mean value was reported as the final antibacterial efficacy.

Cytotoxicity Assessment

The cell viability of the AgCl NPs coated cotton fabrics was examined with rat skin fibroblasts (ATCC CRL-1213) according to the XTT assay method described in ISO 10993-5. All samples were dipped in cell culture medium (DMEM with 10 % FBS) at 37 °C for 24 h with shaking. Rat skin fibroblasts were incubated in DMEM with 10 % FBS at 37 °C in 5 % CO_2 and 95 % humidity. Subsequently, cells were trypsinized and resuspended in culture medium. An aliquot of 100 μ*l* of the cell suspension was seeded in 96-well plates. After incubation for 24 h, the culture media were replaced with liquid extracts of the samples. After another 24 h incubation of the extracts, the XTT reagent (50 μ*l*) was added to each well and the plates were incubated in the dark at 37 °C for another 4 h. The absorbance of each well at 490 nm was measured with a reference wavelength of 690 nm using a microplate reader. Cells incubated in culture medium (without extraction of samples) were tested under the same conditions to serve as negative controls.

Washing Stability Testing

The washing stability of the AgCl NPs coated samples was measured using a Launder-Ometer (Darong Textile Instrument Co., Ltd., Zhejiang, China) according to AATCC Test Method 61-2010 [7,12]. BTCA/AgCl treated cotton swatches were subjected to repeated laundry cycles inside sealed stainless steel canisters containing 150 m*l* of 0.15 % AATCC detergent water solution together with 50 stainless steel balls. In this method, every washing cycle (45 min) with 42 rpm in the Lauder-Ometer at 49 °C was equivalent to five home machine washings. After being washed for desired washing cycles, samples were rinsed with distilled water thoroughly and dried. The antibacterial efficacy against *S. aureus* and the silver content after washing were measured according to the method outlined above.

Physical Property Measurement

Breaking strength in warp direction of the warp -knitted fabrics was measured with an electronic strength tester (YG(B)026D, China) according to the GB/T3923-2013 method. The whiteness of fabrics before and after the treatment was measured by a WSD III whiteness instrument (Yakang optical instrument Co., Ltd., China). Wrinkle recovery angles (WRA) of untreated samples and treated samples were measured according to GB/T3819-1997. Five replicates were performed for the breaking strength and whiteness testing, and the average values were reported. Nitrogen (N_2) adsorption on the surface of samples was measured to calculate the specific surface area using the Brunauer-Emmett-Teller (BET) equation.

Results and Discussion

Characterization of AgCl NPs

Figure 1(A) shows the UV spectra of AgCl colloid produced in this study. The absorption peak exhibited at

260 nm is due to the characteristic absorption property of AgCl NPs [29,30]. In order to further confirm that the produced particles are AgCl, the precipitated powders were collected after 2 days' standing in dark environment and determined by XRD (Figure 1(B)). The characteristic diffraction peaks at 28.06 °, 32.48 °, 46.44 °, 55.02 °, and 57.70 ° are well assigned to the (111), (200), (220), (311), and (222) crystal planes of cubic lattice of AgCl NPs [29-32].

The particle size distribution of AgCl NPs was detected by DLS, and the results were summarized in Figure 1(C). The average size of the NPs in this study is about 120 nm, which is much smaller compared with commercially available AgCl antibacterial agent [27,28]. Smaller particle size is favoring the absorption onto the fiber surface and obtaining better binding force. And the smaller particles with bigger specific surface area could show better antibacterial efficiency than the larger particles [28].

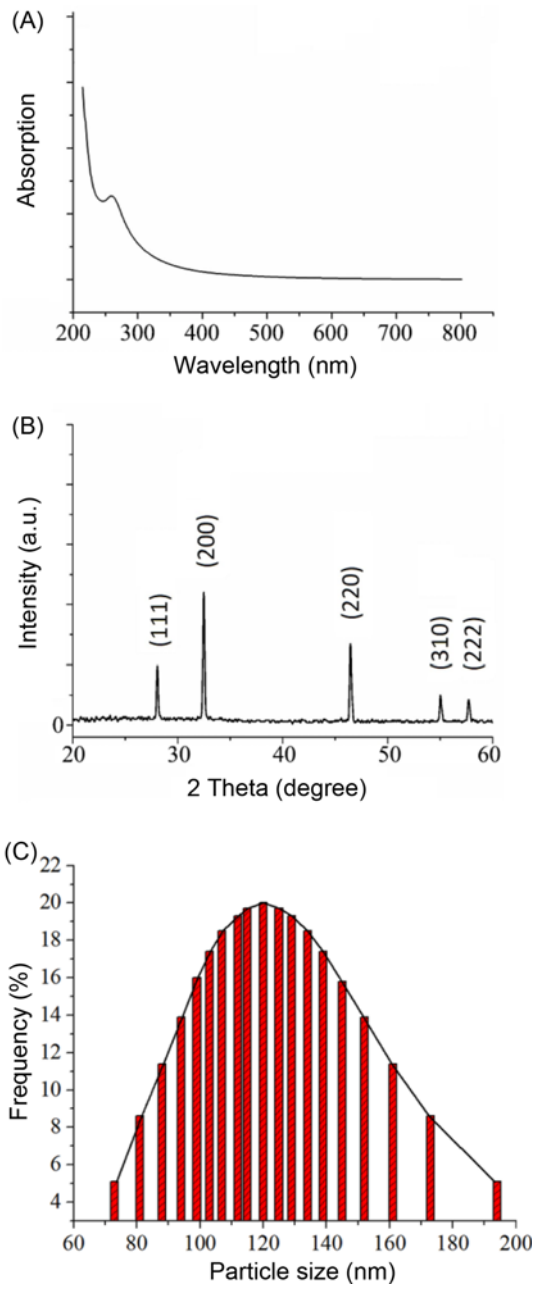

Figure 1. Characterization of AgCl NPs; (A) UV spectra, (B) XRD pattern, and (C) DLS.

Characterization of AgCl NPs Coated Cotton Fabrics

The fourier transform infrared spectroscopy (FTIR) is an effective characterization tool to investigate the structure change of chemically modified cellulose materials [12,13, 25]. The FTIR spectra of untreated and treated samples are shown in Figure 2. Obviously, it can be noticed that a strong peak is presented at 1721 cm^{-1}, which belongs to C=O groups introduced by BTCA [12,13]. This is a piece of evidence confirming the reaction between BTCA and cotton cellulose. BTCA was coated onto fiber surface as a binder to improve the washing stability of AgCl NPs coated cotton fabrics. Owing to low concentration of silver chloride in colloid used in this study, AgCl NPs could not be detected in FTIR spectra. SEM and ICP-OES analysis were used to verify whether AgCl NPs were coated onto cotton fiber.

Figure 3 shows the SEM images of the surface morphologies of the control cotton fibers and modified cotton fibers. Compared with the smooth surface of untreated cotton fibers, the surface of AgCl treated cotton fibers was covered with a large number of NPs. It can be noted that there were many small AgCl NPs with size about 100 nm presented on the surface of the fibers. Also, some agglomerated particles with size about 500 nm were observed in the picture, which might be caused by the aggregation of AgCl NPs. The silver content in the treated cotton fiber was 249 ppm detected by ICP-OES, which was higher than that in treating solution, indicating initiative adsorption of the AgCl NPs onto cotton fibers.

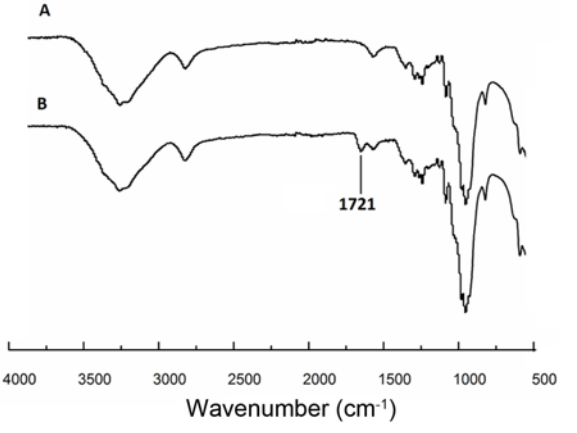

Figure 2. FTIR spectra of untreated (A) cotton sample, and (B) BTCA/AgCl coated cotton sample.

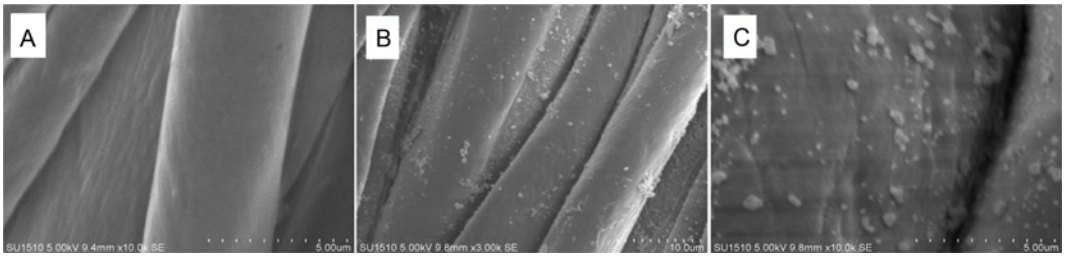

Figure 3. SEM images of (A, 10000×) untreated cotton sample, (B, 3000× and C, 10000×) BTCA/AgCl coated cotton sample.

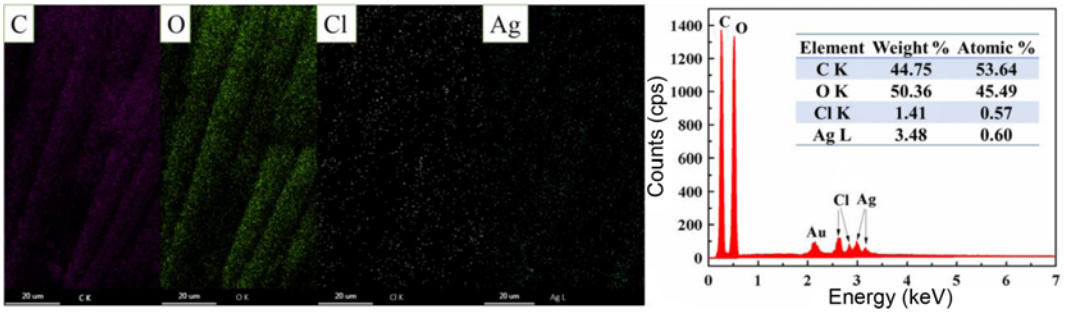

Figure 4. EDX images of BTCA/AgCl coated cotton sample.

The chemical composition on the fiber surface coated with AgCl colloid was illustrated by EDX, as presented in Figure 4. Chloride and silver were detected in EDX pattern, and their mass contents were 1.41 % and 3.48 %, respectively. This result further demonstrated that AgCl NPs was attached onto the surface of cotton fabrics.

Antibacterial Efficacy and Durability

Both Gram-positive bacteria and Gram-negative bacteria were challenged in the antibacterial efficacies testing. The excellent antibacterial function is clearly noticeable in Table 1. The samples coated with AgCl NPs provided 5.2 log and 5.7 log reduction against *S. aureus* and *K. pneumonia*, respectively. The excellent antibacterial function of the fabric is due to the high efficacy of silver ion, which can inactivate bacteria by attacking targets including proteins, nucleic acids, and cell membrane [33]. As a comparison, the bacteria on control cotton samples increased by 2-2.5 log, indicating the control samples are good media for the growing of the two kinds of bacteria under the incubation condition in this study.

Table 1. The antibacterial efficacies

Name of test organism (stain number)	*S. aureus* (ATCC 6538)	*K. pneumonia* (ATCC 4352)
Concentration of inoculum (CFU/m*l*)	1.7×10^5	1.8×10^5
Growth value of control samples F	2	2.5
Growth value of treated samples G	-3.2	-3.2
Antibacterial activity value A (A=F-G)	5.2	5.7

Cytotoxicity Test

Cytocompatibility of the BTCA/AgCl treated cotton sample was investigated using the XTT assay method. As shown in Figure 5, the cell viability of untreated cotton fabrics reached nearly 105 %, which could be explained by that the porous structure of cotton fabrics facilitated cell adherence and created a more favorable environment for cell growth. In contrast, the cell viability of AgCl NPs coated cotton fabrics was about 83 %, indicating that the sample was not cytotoxic and allowed the cell attachment and proliferation.

Washing Stability Evaluation

To evaluate the washing stability of AgCl NPs coated

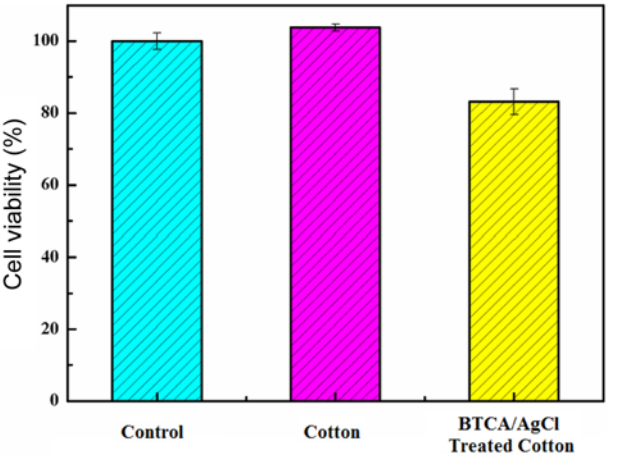

Figure 5. Cell viability of the BTCA/AgCl coated cotton sample.

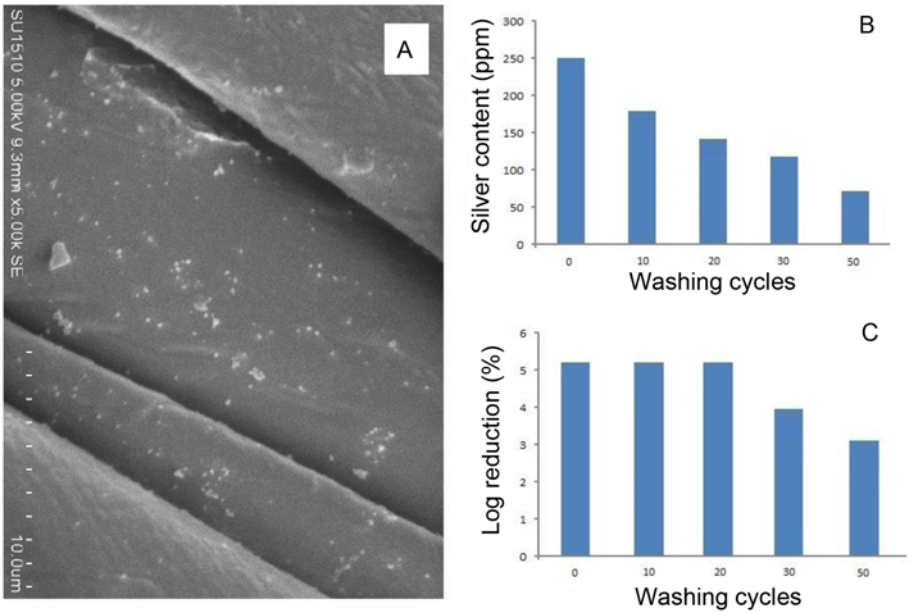

Figure 6. (A) The SEM image of AgCl NPs coated fibers after 50 washing cycles, (B) the silver content of the samples after different washing cycles, and (C) the antibacterial activity against *S. aureus* of the samples after different washing cycles.

fabric, SEM image, silver content and antibacterial test was detected after several washing cycles. After 50 washing cycles, the amount of particles on fiber was reduced compared with unwashed samples. However, many NPs still remained on the surface of cotton fiber (Figure 6(A)). Interestingly, most of the remained AgCl NPs had small particle size about 100 nm. This was because the bigger particles have poor bonding force, and were washed off easily. The remained silver content after different washing cycles was quantitatively detected by ICP-OES as shown in Figure 6(B). After 20 washing cycles, more than 44 % of the silver content was lost. Although the silver content decreased under the action of the detergent in washing solution and mechanical force, there was no obvious change in antibacterial activity of AgCl coated cotton fabrics as shown in Figure 6(C). When the washing time prolonged to 50 cycles, there was still 90 ppm silver content remained on cotton fabrics. According to previous studies, this remaining silver content was sufficient enough to obtain a good antibacterial activity [23-25,28]. In this study, after 50 washing cycles, the antibacterial activity value against *S. aureus* was 3.1 log, which could be interpreted that the washed samples still maintained strong antibacterial property.

Physical Property Evaluation

Obvious color change on traditional silver NPs modified fabric is an undesirable side effect. But in our study the AgCl NPs only caused a slight decrease on fabric's whiteness as shown in Table 2. This is an advantage of AgCl used in antibacterial treatment of fabric [23-25,28]. The WRA of AgCl NPs coated cotton fabrics was significantly

Table 2. Results of physical property evaluation of cotton fabrics

Sample	Whiteness (%)	WRA (°)	Breaking strength (N)	Specific surface area (m^2/g)
Untreated	93	120	551	5.1127
Treated	85	260	339	33.3952

increased from 120 ° to 260 ° due to the formation of cross-linking between cotton cellulose and BTCA [12]. The breaking strength of AgCl NPs coated cotton fabrics was reduced by about 38 % because of the degradation of cellulose macromolecules caused by BTCA treatment, which was common in the functional finishing of textiles. The advantages in whiteness and wrinkle recovery of BTCA/AgCl NPs coated fabric could increase the potential value of this method. After BTCA/AgCl NPs treatment, the specific surface area of the cotton samples increased significantly (from 5.1127 to 33.3952), which has a positive effect on the antibacterial activity.

Conclusion

In this work, a simple process was used to coat AgCl NPs onto cotton fabrics with the cross-linking agent BTCA. The AgCl NPs treated cotton fabrics exhibited potent and durable antibacterial efficacy against *S. aureus* and *K. pneumonia*. Meanwhile, the biocompatibility of the coated cotton fabrics was acceptable according to the cell viability. After 50 washing cycles, the samples still maintained enough silver content to keep good antibacterial activity. In

addition, this treatment had little effect on the whiteness of cotton fabrics, and could improve the anti-wrinkle properties of cotton fabrics significantly with the WRA increasing from 120 ^{o} to 260 ^{o}. The traditional pad and dry equipment used in textile industry can meet the requirement of this method, and the AgCl NPs treated cotton fabrics have potential applications in the biomedical textiles.

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