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Article

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In Situ Synthesis of Silver Nanoparticles for Ag-NP/Cotton Nanocomposite and Its Bactericidal Effect

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For years, nanotechnology has been considered as an important field that has opened new opportunities for extensive research. In biomedical applications, of all the metal nanoparticles, silver nanoparticles (Ag-NPs) have played an important role because of their antibacterial properties. Ag-NPs have been demonstrated to possess antibacterial properties in many applications. However, the minimum number of NPs required on the surface to prevent bacterial growth is yet to be determined. It is worthwhile studying the decrease of bacterial growth rate or the level of inhibition as a function of the size or density of NPs. Therefore, in this paper we discuss the size of the NPs that can stimulate the bactericidal property. It should also be noted that NPs larger than 100 nm might not be effective against bacteria. Moreover, this study employs polyvinyl pyrrolidone (PVP) and cellulose as reductants to form strong covalent bonds under UV light, which can help synthesize Ag-NP/cotton nanocomposites. This type of nanocomposite displays high cell viability and improved antimicrobial activity. A fairly simple application involves the use of UV light to increase particle distribution and impart bactericidal property.

Keywords: Silver nanoparticles; Ag-NP/cotton nanocomposite; Bactericidal effect; Toxicity.

INTRODUCTION

Conventional polymers and fibers do not provide resistance against microorganisms or materials generated through their metabolism. In most cases, they are prone to proliferation, accumulation, and multiplication of microorganisms in their environment surrounding them. In addition, various factors such as favorable humidity and temperature and the existence of materials on the textile surfaces make the environment similar to optimal enrichment cultures that allow rapid multiplication of microorganisms.[1–3] Thus, controlling these unpleasant factors is crucial to improving hygienic living standard. The focus of many research studies has been on antibacterial modification of textiles.[4]

Currently, the application of nanosized organic and inorganic nanoparticles (NPs) in medical devices has increased because of their ability to become biologically functionalized. Antimicrobial agents are used in numerous applications in medical care, healthcare, environmental products, and synthetic textiles. These agents can be categorized into two: organic and inorganic.[5] Over the past decade, inorganic materials have gained much popularity because of their ability to tolerate adverse processing conditions. The antibacterial

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activity can also be a function of the surface area that is in contact with the microorganisms. Therefore, a broader range of probable reactions with the presence of bioorganics on the cell surface, such as environmental inorganic and organic species, can be seen with a larger surface area (as in NPs).[6]

Among metals (oxide), silver nanoparticles (Ag-NPs) show the strongest antimicrobial activity. The synthesis of Ag-NPs is driven by their diverse applications in the fields of clinical therapeutics, biomedical diagnosis, electronics devices, sensors, pharmaceutical, alimentations, print instruments, and many other fields.[7,8] Among all the known procedures, a simple and quick method would be the green reduction chemical pathway, which does not require the use of complex equipment.[5,9] In this manner, physiochemical characteristics, such as localized plasmonic properties, are influenced by factors like morphology and uniform dispersion of NPs, which must be controlled.[10–12] To resolve the target problem, surfaces of cotton fabrics have been coated with colloidal NPs both technically and chemically.[13–15] As a potential platform in bactericidal applications, investigation of cellulose has been done to determine its benefits as a nanoreactor and stabilizer.[1,16] In an attempt to extend the study, other researchers have designed the grafting approach to improve cytotoxicity.[4,17] Since NPs exert a cytotoxic effect on human cells, many ideas were proposed to synthesize NPs by forming covalent bonds.[16,18,19] A recent study has shown that thiol-modified cellulose fabrics provide an excellent platform to establish efficient bonding between cotton and metal NPs. The treatment was evaluated by comparing these with untreated NP cellulose conjugates, which revealed that strong conjugates of nanocomposites are created as a result of the presence of the thiol group. Various methods have been employed to functionalize Ag-NPs on diverse fabrics. Some researchers have employed the patented ion-beam-assisted deposition process (developed by Spire Corporation) to synthesize Ag/poly(ethylene terephthalate) fabric (Meadox double velor) coated with metallic silver.[20] Other reported methods include the use of constant pressure padding and of isopropanol or ethanol for the reduction of silver ions. A few methods involve reactions in a liquid medium. The synthesis of Ag-NPs requires surfactants, reducing agents, or templates. However, this method also has a few disadvantages related to the environment effects. Sonochemical irradiation is another effective method employed in the synthesis of nano-phased materials and for the deposition and insertion of NPs into/onto mesoporous ceramic and polymer supports.[21] Another previous work used nano-silver to coat nylon chips.[22] Our aim was focused on sidestepping the fiber production stage to facilitate the development of a direct method to coat commercial fabrics with Ag-NPs.

In our case, we used UV light to synthesize NPs directly on the cotton surface (*in situ* synthesis). As a reductant, we employed the cellulose cotton fiber, while introducing poly(vinyl pyrrolidone) (PVP) to form strong covalent bonds.

RESULTS AND DISCUSSION

Fourier transform infrared (FTIR) spectroscopy was used to characterize the Ag-NPs/cotton nanocomposites with different concentrations. As seen in Figure 1, all three samples were found to be identical based on the FTIR spectrum of the Ag-NP/cotton nanocomposites. The sharpest peaks for free OH were found between 3375 and 3410 cm^{-1}. The carbonyl groups of PVP (C=O) displayed strong peaks from 1600 to 1650 cm^{-1}, the C–H bonds from the aromatic cycles of cellulose have a peak at 3000 cm^{-1}, the C–O group from cellulose revealed high fluctuation around 1200 cm^{-1}, the C–N groups from PVP presented a small peak in the region 1420–1460 cm^{-1}, and the C–H bonds of PVP (similar to alkane) showed a peak around 700 cm^{-1}. The vibration of C–O from cellulose and C–N from PVP signify that these two molecules are involved in Ag-NP coordination. It shows the sharing of the atom between O from the alcoholic group in cellulose and Ag. This reaction has a high impact on the absorbance of silver particles. However, such a reaction is also observed between N and Ag. The redshift of C–N bond also confirmed the result.

Many nanomaterials have been reported to be potentially harmful, as they influence the organisms on subcellular fraction at the cellular and protein levels. As nanomaterials have a high surface area to volume ratio, cell toxicity becomes a significant concern. As observed above, the strong adhesion between cotton fibers and Ag could help prevent leaching of Ag-NPs from the cotton network and entering the organisms, which is essential for the biomedical application of Ag-NP/

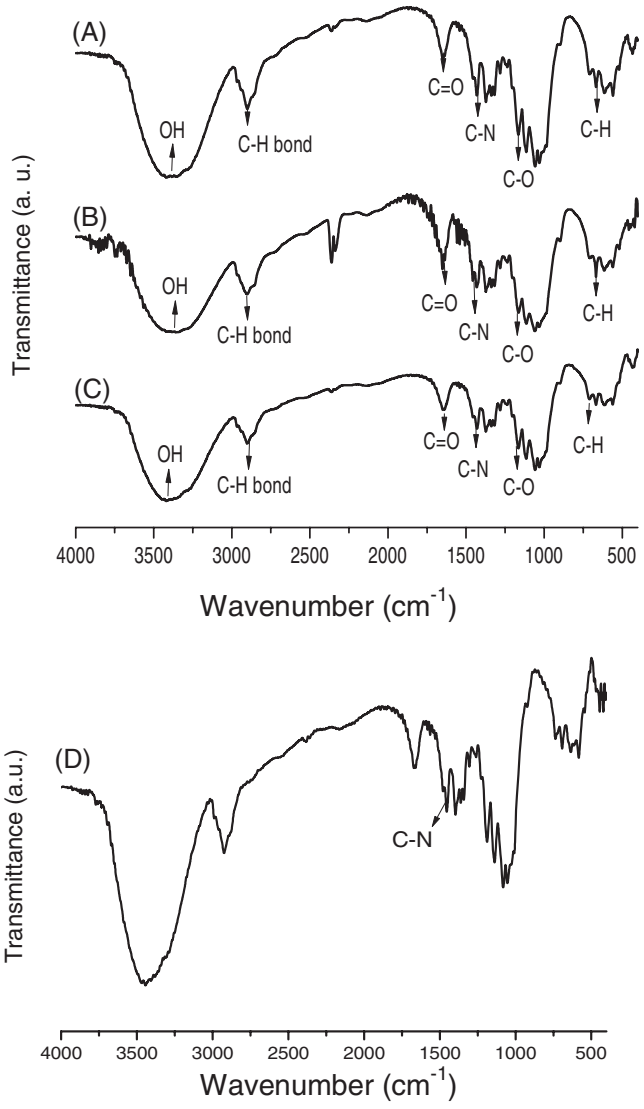

Fig. 1. FTIR spectra of sample A (0.005 M), B (0.01 M), C (0.05 M), and D (cotton).

cotton nanocomposite. Our hypothesis is supported by cytotoxicity tests.

Scanning electron microscopy (SEM) images from Ag-NP/cotton nanocomposite and pure cellulose fibers of the cotton fabric are presented in Figure 2 to analyze the spreading of Ag-NPs on the cotton fabric fibers. The result reveals the dispersion of Ag-NPs along cotton fibers without any aggregation. A higher magnification SEM image (in Figure 2(b)) presents a better view, allowing the measurement of the size of the synthesized Ag-NPs on cotton fibers.

Figure 2(c) shows the energy-dispersive X-ray spectrum (EDS), which shows the formation of Ag NPs on the cellulose cotton fiber surface. The peak of Ag in the EDX spectrum shows the significance of Ag in the Ag-NP/cotton nanocomposite.

In addition, the transmission electron microscopy (TEM) images presented in Figure 3 support the formation of Ag-NPs on cotton fabric's cellulose fibers. All Ag-NPs formed on the fibers showed identical nature and were spherical in shape. The average size of the formed particles for sample A ($AgNO_3$, 0.005 M), sample B ($AgNO_3$, 0.01 M), and sample C ($AgNO_3$, 0.05 M) was 25 ± 5, 30 ± 5, and 40 ± 5 nm, respectively. Compared to the average size of the formed Ag-NPs in the lower molarity $AgNO_3$ stock solution, that of Ag-NPs in higher molarity $AgNO_3$ stock solution was larger. The TEM images clearly show that the Ag-NPs do not aggregate. Accordingly, the surfaces of the Ag-NPs are affected by the strong interaction between the nitrate group ($-NO_3$) of the $AgNO_3$ stock solution and the hydroxyl groups ($-OH$) of cellulose molecules of cotton fabric.

The growth of *Escherichia coli* can be inhibited by Ag-NP/cotton nanocomposite, which also displays the best antimicrobial properties in solid (Figure 4) and liquid media (Figure 5).

As can be clearly seen in Figure 4, samples A and B failed to stop bacterial growth even though the growth was very slow. However, bacterial growth was stopped to a significant level in sample C.

The results regarding the antimicrobial properties of samples in a liquid medium are shown in Figure 5. It is clear that sample C showed more antibacterial activity compared to the other samples because the concentration of $AgNO_3$ in sample C is higher, thus resulting in a higher density of Ag-NPs on the cotton fabric. As a general rule, with an increase in the incubation time from 3 to 28 h, the antibacterial activity should decrease for Ag-NP nanocomposite. After 12 h of incubation time, the highest antibacterial activity could be observed for all samples. Overall, the results demonstrate that the Ag-NP nanocomposite shows high activity, which can be lowered or raised by varying the molarity of $AgNO_3$ solution. The result was also confirmed by quantitative antimicrobial analyses (AATCC 100). The results indicated 98% reduction for the Ag-NP/cotton nanocomposite in the presence of *E. coli* bacteria (Table 1).

Figure 6 shows the fluorescent microscopy image showing the growth of human skin fibroblasts (HSFs)

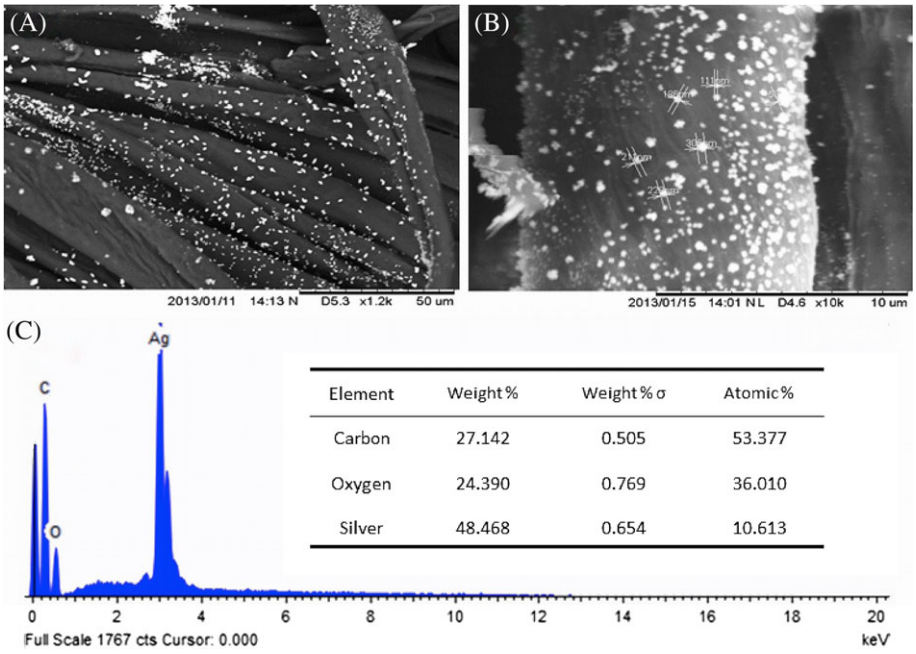

Fig. 2. SEM images of Ag-NP/cotton nanocomposite (sample C (0.05 M)) with different magnifications (a) ×1200 and (b) ×10 000. (c) EDS image from Ag-NP/cotton nanocomposite.

with Ag-NP and Ag-NP/cotton nanocomposite treatment, 2 days post seeding. For the Ag-NP/cotton nanocomposite, the cell culture shows improved proliferation and cell viability. This supports the strong adhesion between the cotton fibers and Ag, which may help in preventing leaching of Ag-NPs from the cotton network and entering of the organisms. This may prove crucial in biomedical application of the Ag-NP/cotton nanocomposite. Our hypothesis is supported by cytotoxicity tests and the subsequent results of Ag release. On the other hand, cell viability is not shown by Ag-NPs, as it can result in the production of reactive oxygen species (ROS). There were chances that the cell wall of HSFs could facilitate the distribution of ROS.[23] Table 2 shows the release of the inorganic content from the cotton of Ag-NP/cotton nanocomposite. Based on Table 2, Ag-NP/cotton nanocomposite shows a low amount of inorganic release from the cotton. Therefore, this also confirmed the presence of strong adhesion between cotton fibers and Ag.

EXPERIMENTAL

Materials and methods

The materials employed in this study included PVP (QReC), Ag-NPs, cotton, sodium carbonate (Na_2CO_3, QReC), and 100% cotton white plain weave cotton (Mirota Batik, Surabaya, Indonesia, unmercerized, bleached, with 126 denier, or 14 mg/m and a fabric count of 95 × 95, having a mass density of 9.3 mg/cm^2, and 160 fibers/in.).

Gram-negative bacteria *E. coli* (strain.DH5D-*E. coli*) were employed in Luria Bertani (LB) medium (Himedia Laboratories Ltd) to conduct bactericidal experiments. Tryptone or peptone (Sigma Aldrich), agar (Sigma Aldrich), and yeast extract (Sigma Aldrich) were also employed in the experiment. HSF (1184 catalog no.90011883, available from ECACC, UK) was used in the cytotoxicity test. Other materials employed include fetal bovine serum (FBS, Sigma Aldrich), the minimum essential media (MEM) (catalog no. 11095, Invitrogen), phosphate-buffered saline solutions (PBS, Sigma Aldrich), penicillin–streptomycin (PS) (Sigma Aldrich), Hank's balanced salt solution (HBSS, Sigma Aldrich) trypsin/EDTA (Invitrogen), and ™Red CMTPX dye (Sigma Aldrich).

Ag-NP/cotton nanocomposite

For scouring cotton fabrics, a solution of 0.19 M of Ag-NPs was employed to immerse 100 g of cotton. The solution contained PVP as a stabilizing agent for Na_2CO_3 and original cellulose of the cotton fabrics as a

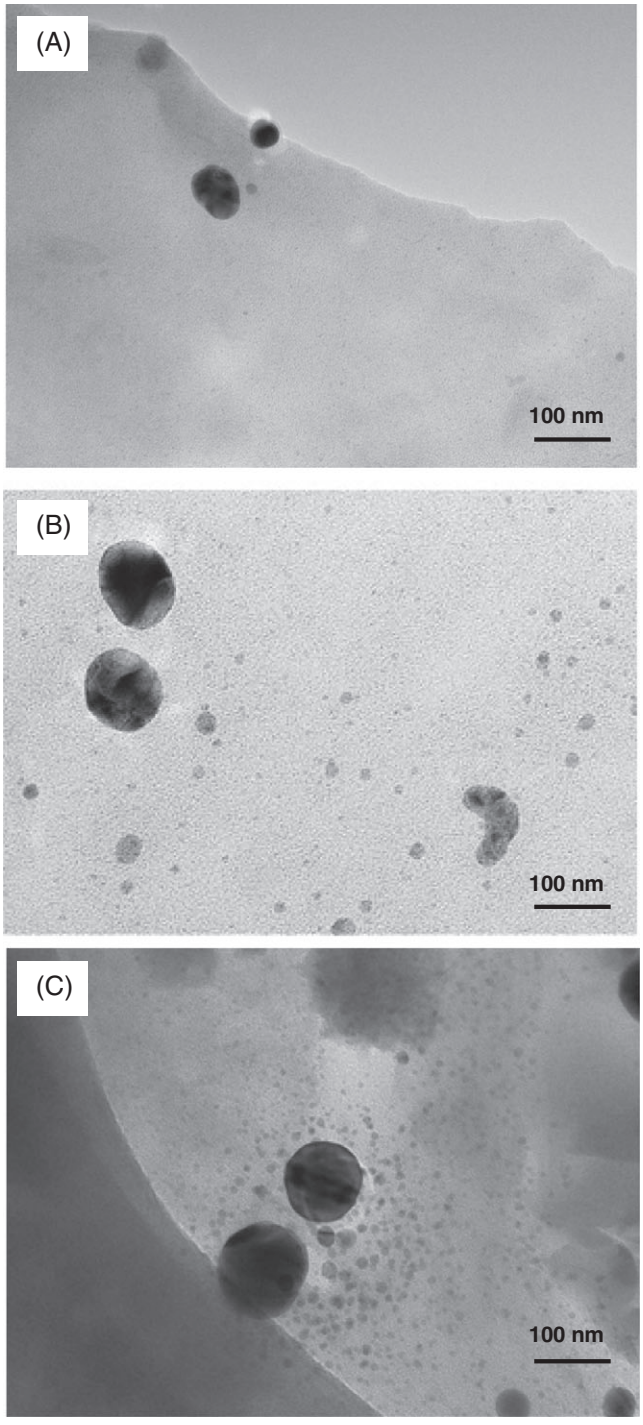

Fig. 3. TEM images of samples A (0.005 M), B (0.01 M), and C (0.05 M).

reducing agent. The solution sample was then exposed to UV radiation for 2 h. For *in situ* synthesis agent, to evaluate the appropriate protocol, silver nitrate and PVP with five different concentrations were prepared.

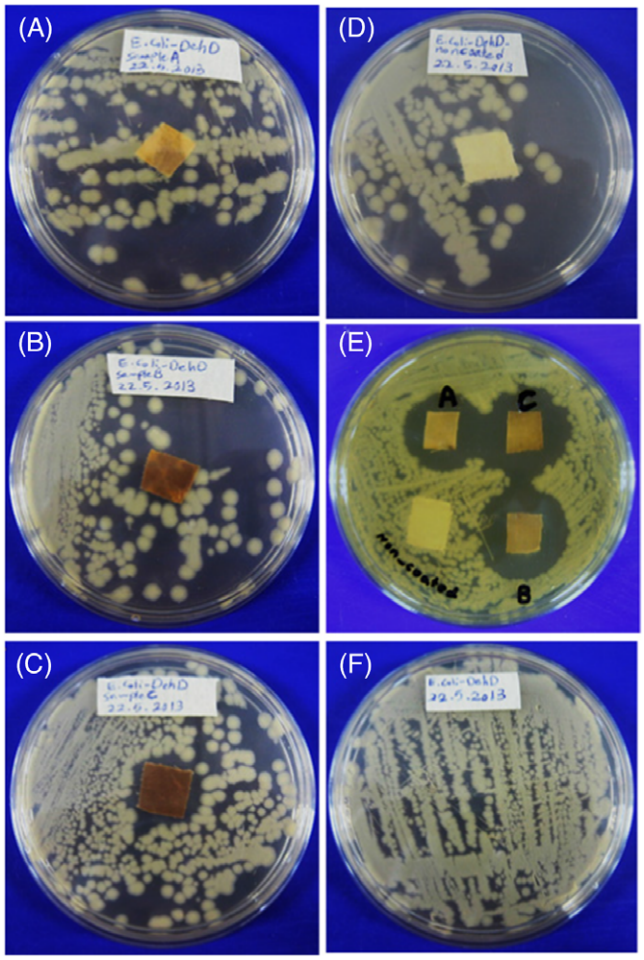

Fig. 4. The inhibition of *E. coli* colonies on solid Luria medium against (A) sample A, (B) sample B, (C) sample C, (D) uncoated sample (E) samples A, B, and C vs. the uncoated sample and (F) culture *E. coli*.

$AgNO_3$ stock solutions (0.0080, 0.0050, 0.01, 0.03 and 0.05 M) were prepared as well. Table 3 presents the preparation of all samples.

Material characterization

A Nexus 670 spectrometer (Nicolet, USA) was employed to perform FTIR spectroscopy to distinguish the structural features of heat-treated powders. Measurements were conducted in the range 4000–400 cm^{-1}. A TEM (HT7700 Hitachi) and an SEM (JEOL JSM-6380LA) equipped with an EDS system (JEOL Inc., Tokyo, Japan) were employed to analyze microstructures.

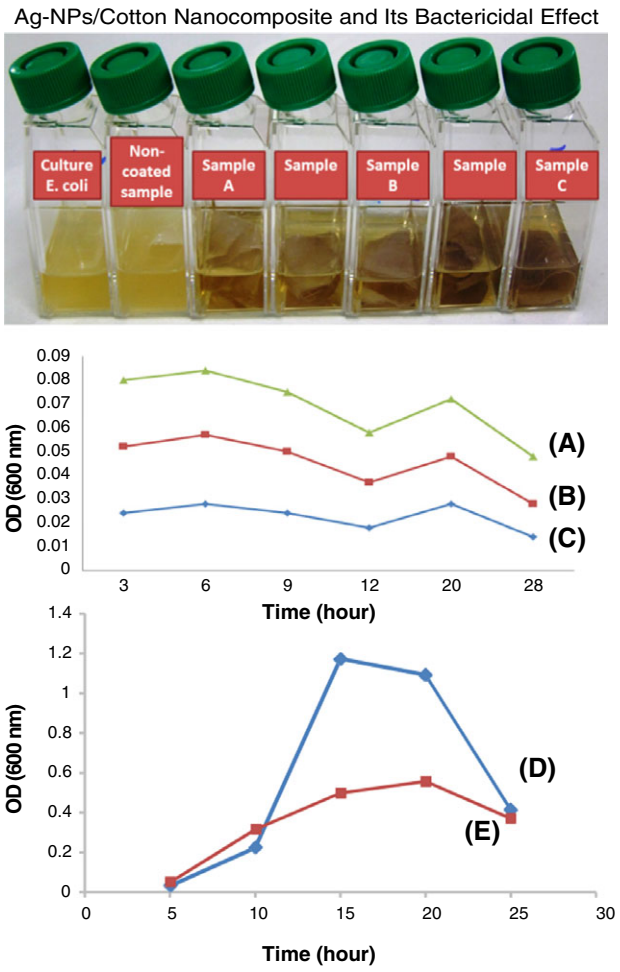

Fig. 5. Optical density measurement of (A) sample A, (B) sample B, (C) sample C, (D) culture *E. coli*, and (E) uncoated cotton by using UV spectroscopy.

Table 1. Antibacterial properties of Ag-NP/cotton nanocomposite (sample C) according to AATCC 100 test method

Sample	Reduction (%)
Ag-NP/cotton nanocomposite (sample C)	98%
Uncoated	No growth reduction

Antibacterial activities of Ag-NP/cotton nanocomposite

E. coli growth on solid and liquid Luria media was screened to confirm the bactericidal effect of Ag-NPs on cotton fabrics. The preparation of liquid Luria involved dissolving of tryptone/peptone (5 g), sodium chloride (5 g), and yeast extract (2.5 g) to make the final volume of 500 mL with distilled water. For media solidification, 7.5 g of agar powder was mixed to the final solution. Then, the apparatus, media, and non-coated fabrics all were autoclaved.

E. coli cells were then cultured in liquid and on solid media following sterilization. In each tube, 30 mL of medium was placed. Also, simple cultures of *E. coli* cells were mixed in the samples with the uncoated cells with sample 1 coated; sample 4 coated cotton fabrics, and sample 5 coated cotton fabrics; and cells with sample 2 coated and cells with sample 3 coated cotton fabrics. For all tests, 155 mg of cotton pieces were used. The temperature was kept at 37°C, and the sample cultures were maintained with shaking to allow circulation of the whole media with cotton surfaces. The UV spectroscopy at 600 nm was employed to measure optical density periodically. The development of *E. coli* colonies were seen in solid Luria medium with an uncoated pattern, sample 1, sample 2, sample 3, sample 4, and sample 5. The samples were screened four times within the duration of 2 h. Then, after 24 h, the final measurement of optical density was done. The culture was then allowed to stay overnight at 37°C. The antimicrobial activity of Ag-NP/cotton nanocomposite was also shown by AATCC 100 protocol. Equation (1) was used to calculate the percentage reduction:

$$\text{reduction}\% = (B - A)/B \quad (1)$$

where A is the number of bacteria recovered from the inoculated Ag-NP/cotton nanocomposite over desired contact period, and B is the number of bacteria recovered from the inoculated, uncoated cotton. Zero percent reduction indicates no antimicrobial efficacy, and 100% reduction indicates complete antimicrobial efficacy.

Cell culture test

HSF cells (12 mm cell size) were used to evaluate the cytotoxicity effect of Ag-NPs and Ag-NP/cotton nanocomposites. The Freshney protocol was followed to culture HSF cells.[24] The cells were then cultured in MEM with 10% (v/v) FBS, 2 mM glutamine, and 1% (v/v) PS. In a humidified incubator (5% CO_2 at 37°C), the attached cell cultures were preserved at the specified concentrations of 2–9 × 10^5 cells per mL. Within 72 h, the cells reached the confluence stage. The cells

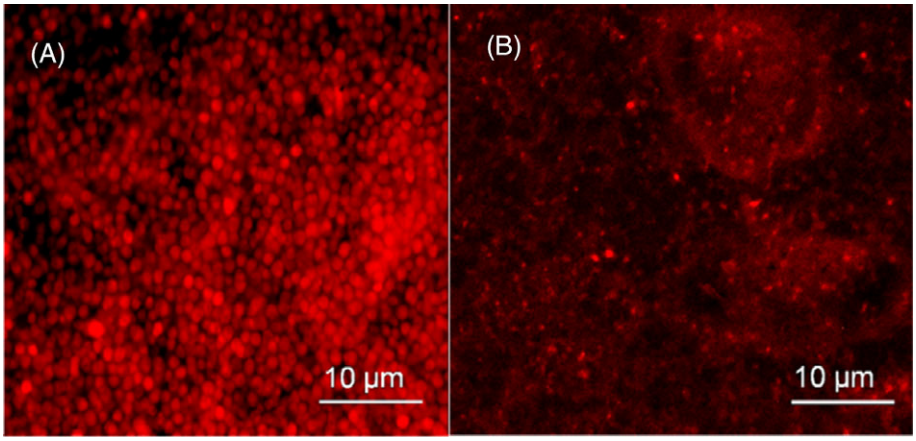

Fig. 6. Fluorescent microscopy image of human skin fibroblast (HSF) growth on the different treatments: (a) Ag-NP/cotton nanocomposite, (b) Ag-NPs.

Table 2. The release of the inorganic contents from the cotton of Ag-NP/cotton nanocomposite

Sample	Inorganic release amount (μg/L)
Ag-NP/cotton nanocomposite (sample C)	3.018

Table 3. Preparation of all samples

Sample	$AgNO_3$ (original Ag source) (mg)	PVP (surfactant) (mg)	Scoured cotton (reductant) (mg)
1	0.059	10	155
2	0.095	15	
3	0.118	250	
4	0.356	350	
5	0.694	500	

passages employed were P11–P15. When the cells were about 80% confluent, PBS was used to wash the cells. Later, 0.25% trypsin/EDTA was employed to detach PBS. The cells were then centrifuged at 2100 rpm for 5 min to obtain the cell pellets. The obtained cell suspensions were employed in 3 mL of MEM containing a cell concentration of 5×10^5 cells per mL. Finally, ™Red CMTPX dye was used to stain the cells. The HSF cells were placed in each well of a 12-well plate without or with samples (Ag-NPs and Ag-NP/cotton nanocomposite), 24 h before each experiment.

Metal release analysis

Ag-NP/cotton nanocomposites were placed in distilled water (20 mL) for 14 h, after which the nanocomposite was removed by centrifugation. The release of the inorganic content from the cotton was analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

CONCLUSION

A simple and new method that employs PVP was used to synthesize Ag-NP/cotton nanocomposites. Cellulose was used as the reductant, and PVP to form strong covalent bonds. The result of HSF cell culture indicated the strong adhesion between cotton fibers and Ag, which may help in preventing the leaching of Ag NPs from cotton network and entering of organisms. The growth of *E. coli* could be inhibited by Ag-NP/cotton nanocomposite. These NPs are known for their best antimicrobial properties in liquid and solid media for low surface concentration of Ag-NP provided that the size of NPs is less than 100 nm. However, the cell viability was found to improve with this nanocomposite. Therefore, the remarkable features of the results recommend the use of this easy and environmentally friendly preparation method in biomedical applications. Further studies are needed to evaluate the impact of the Ag-NP/cotton nanocomposite on various other biological molecules used in biomedical applications.

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