

# Cost-Effective Fabrication, Antibacterial Application and Cell Viability Studies of Modified Nonwoven Cotton Fabric

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## Research Article

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# Abstract

Nonwoven cotton fabric has been fabricated and designed for antibacterial applications using low cost and ecofriendly precursors. The treatment of fabric with alkali leads to formation of active sites. The surfaces were dip coated with silver nanoparticles and chitosan. The surface was chlorinated in next step to transform amide (N-H) groups in chitosan into N-halamine (N-Cl). The modified and unmodified surfaces of the nonwoven cotton fabric have been characterized by FTIR, SEM, and XRD. The active chlorine loading is measured with iodine/ sodium thiosulphate. The antimicrobial activity and cell toxicity assay were carried out with and without modifications of nonwoven cotton fabric. The antimicrobial efficacies of loaded fabric were evaluated against four bacterial species (*Micrococcus lutes*, *Staphylococcus aurea*, *Enterobacter aerogenes*, and *E.coli*). It was found that modified fabric exhibited superior efficiency against gram-positive and gram-negative bacterial strains as compared to their bulk counterparts upon exposure without destroying and affecting fabric nature. The overall process is economical for commercial purposes. The modified fabric can be used for antimicrobial, health, and food packaging industries, and in other biomedical applications.

## Introduction

A large number of health care applications have introduced recyclable antimicrobial wound dressings and biomedical fabrics (Ma, Li et al. 2019). Antimicrobial activity for a long period, stability, and fast disinfection capability with time are defining factors that decrease resistance properties of these materials against microbes. (Yin, Wang et al. 2020) The medical industry including carpets, beds, accessories, and other allied items are facing some serious issues of bacterial contamination of fabrics which may cause severe infections in patients and workers (Goyal, Khot et al. 2019). Hospitals' wearable accessories including lab-coats and costumes are the main shields against bacteria, but these articles often do not offer sufficient antimicrobial properties (Gupta, Bairagi et al. 2016; Zhang, Kai et al. 2019). Therefore, the modified inexpensive, biodegradable, rechargeable and recyclable, less toxic, durable, and eco-friendly antimicrobial wound dressings and fabrics are needed to be developed. This could potentially be achieved by incorporating stable and non-bleached *N-halamine* into the materials as biocides. (Demir, Broughton et al.). Such materials have been used owing to their good stability, regenerate ability and high efficacy to inactivate bacteria. (Chen, Yu et al. 2017) Cotton cellulose based materials are highly suitable objects for the growth of bacteria but can be functionalized in a facile way to enrich with growth inhibition properties. The wet finishing or grafting, layer by layer assembly, and dip coatings techniques are commonly used to produce a stable cotton fabric with enhanced antibacterial resistance properties. (Liang, Chen et al. 2007; Wang, Huang et al. 2020) The previous studies showed that *N-halamine* effectively forms a strong N-X (Br, Cl, I) bond through covalent interactions at the fabric surface and prevents leaching of halogen atoms in oxidative state of +1. It is well known that the antibacterial action of *N-halamines* is due to the positive halogens from *N-halamines* that are transferred to suitable receptors in microbe cell. In this way, enzymatic or metabolic cell processes are inhibited and consequently killing bacteria and other parasites. (Dong, Wang et al. 2017; Zhang, Li et al. 2020) *N-*

*halamines* are promising functionalities that provide immediate bacterial inhibition against a broad range of microorganisms without affecting the external and internal environment of host. (Yao, Gao et al. 2016; Dong, Wang et al. 2017) *N-halamines* modification of fabric surfaces has been done by different methods including grafting, (Liu and Sun 2008) layer by layer assembly, (Cerkez, Kocer et al. 2011) and dip coatings (Tudu, Sinhamahapatra et al. 2020). *N-halamines* functionalization is carried out at nitrogen-containing surfaces containing imides, amides, or amines through halogenations (X= Br, Cl, I). The cotton cellulose modified with nitrogen-containing monomers and successive chlorination provides prompt inactivation against various bacterial strains (Ren, Kou et al. 2008; Liu and Sun 2009; Ren, Akdag et al. 2009). Chitosan is a naturally existing polymer and has special characteristics like biodegradability; non-toxicity, cationic nature, and antimicrobial activity as well as incorporated with NH<sub>2</sub> groups (Rajoka, Zhao et al. 2019) (Hussain, Musharraf et al. 2019). The NH<sub>2</sub> groups in chitosan are transformed easily into *N-halamines* upon treatment by strong oxidants like household bleach. Treated fabric then acquires powerful antibacterial efficacy. (Abate, Ferri et al. 2019) The aforementioned activity is due to the release of positive chlorine atoms upon dissociation of *N-halamines* on interaction with microbes. (Dong, Wang et al. 2017; Chylińska, Kaczmarek et al. 2019) The active chlorine contents thus released can be recharged and reloaded with chlorine from successive bleach treatments. The regeneration of surfaces is low cost and very simple. It has been reported that the biocidal efficiency of *N-halamine* materials largely depends upon active surface area (Chylińska and Kaczmarek 2020). The large active surface area provides more active sites and is responsible to capture more bacteria. Desirable surface area is obtained by incorporating various substrates like nanomaterials into dressings, fabrics and packing materials along with *N-halamines*. (Tsao, Williams et al. 1991; Li, Li et al. 2013). Nanomaterials not only inhibit or retard microbial growth but also improve the shelf-life of antimicrobial materials. (Ma, Li et al. ; Zheng, Setyawati et al. 2018; Anand, Unnikrishnan et al. 2019). In nanotechnology, nanomaterials like silver, copper, and gold are distinctively important due to their special chemical and biological activities. (Hossain, Perales-Perez et al. 2014; Gupta, Mumtaz et al. 2019). Metallic silver and silver-based nanomaterials are popular agents used a long time in different health care products to enhance antibacterial effectiveness and to reduce infections. (Shahid ul, Butola et al. 2016; Fei, Ma et al. 2020). Significantly at the nano-scale, silver materials show exceptional physical, chemical, biological properties than other bulk materials and are non toxic (Butola and Mohammad 2016). Silver materials exists in different oxidation state (0,+1,+2,+3). The antimicrobial activity of the silver substrates is only depends upon the bioavailability of silver in +1 state. The silver ions in +1 oxidation state slowly releases from the substrate and interact with microorganisms at the cellular level, releases and effectively damage cellular material. (Li, An et al. 2019). The sufficient availability of silver ions, water solubility in body fluid of microorganisms, and longevity of antimicrobial activity are key challenges and limitations. Currently, researchers have been focused to develop a perfect antimicrobial dressings by using *N-halamine* biocides.

Different kinds of *N-halamine* compounds have been extensively investigated for antimicrobial activities.. (Zhu, Chang et al. 2019) Inspired by earlier findings, this paper aims to investigate nonwoven cotton fabric coated with *N-halamine* and silver nanoparticles to enhance synergistically their

effectiveness against microbial growth. *N-halamine*, chitosan, and silver nanoparticles modified nonwoven cotton fabric (hereafter termed and read as *N-halamine-chitosan@AgNPs* (NWCF)) are fabricated by the chlorination of chitosan coating. The chlorinated chitosan and silver nanoparticles nonwoven cotton fabric is evaluated for its antimicrobial activity. The fabric is also tested for cell toxicity assays against *HeLa Cell* Lines. The uniqueness of the present modified method is that fabric surfaces can easily be reactivated, modified, and reproduced by simply treating them with sodium hypochlorite. The fabric also shows stability for a longer time.

## Experimental

### Reagents

Silver nitrate ( $\text{AgNO}_3$ ), Ascorbic acid ( $\text{C}_6\text{H}_6\text{O}_8$ ), Potassium hydroxide (KOH), and Sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) purchased from Merck. Sodium hypochlorite (12.5% Solution), Hydrochloric acid (HCl), Potassium Iodide (KI), and Acetic acid ( $\text{CH}_3\text{COOH}$ ) was obtained from Sigma Aldrich. Nonwoven cotton fabric (bleached and cleaned) was used as a substrate for coating. Chitosan ( $\text{C}_{56}\text{H}_{103}\text{N}_9\text{O}_{39}$ ) was purchased from Junsei Chemical Co. Ltd. Non-ionic detergent (Triton X-100) was used for fabric cleaning.

### Cleaning and Activation of Nonwoven Cotton Fabric

The fabric was obtained from the local market and was treated as the methods described earlier with slight modifications. 1 cm×1 cm dimensions piece of nonwoven cotton fabric were cut, clean, and boiled in 50 mL hot water (100 °C) for 30 minutes using non-ionic detergent (Triton X-100). The fabric was then washed several times with hot water followed by cold water and dried in an oven at 90 °C. The dried fabric samples were stored at room temperature in desiccators and then used for further treatment and characterization.

For activation of fabric surface, pieces of dried nonwoven cotton fabric were soaked in 30 ml potassium hydroxide (6M) solution for 30 minutes at room temperature with an occasional mechanical stirrer. The alkali-activated nonwoven cotton fabric was rinsed several times with distilled water, then padded through a laboratory wringer at low-pressure settings and kept in wet condition until the next treatment. The resulting modified non-woven cotton fabric was named and abbreviated as Nonwoven Cotton fabric (NWCF).

### Surface Modification of Activated Nonwoven Cotton Fabric with Silver Nanoparticles

Further surface modification of activated nonwoven cotton fabric with silver nanoparticles was carried out by following the previously reported method with slight modifications. Briefly, the activated nonwoven cotton fabric piece was immersed in freshly prepared 0.04M silver nitrate solution, stirred for 30 minutes at room temperature. Fabric was taken out from the solution, washed thoroughly with distilled water several times to remove excessive unabsorbed silver ions. Silver ions on the fabric were then reduced using a reducing agent using 0.02M ascorbic acid solutions and stirred again for 30 minutes at room

temperature. Ascorbic acid solution turned clear to milky which indicated the formation of silver nanoparticles. Silver coated nonwoven cotton fabric taken out, washed with distilled water, then padded through a laboratory wringer at low-pressure settings, dried at 65 °C for 90 minutes in the oven. The obtained dried fabric was labeled as silver nanoparticles modified nonwoven cotton fabric (AgNPs-NWCF). The control nonwoven cotton fabrics for comparisons were prepared under the same conditions without the addition of the silver precursor solution.

### **Surface Modification of AgNPs-Nonwoven Cotton Fabric with Chitosan**

Surface of AgNPs-NWCF with chitosan was modified by dip coating method.(Cheng, Ma et al. 2014; Xing, Xu et al. 2016) Simply, 2g of chitosan (Molecular weight:1526.5g/mol.) added in 100 mL of 3% CH<sub>3</sub>COOH aqueous solution at room temperature and vigorously stirred for 30 minutes to obtain a final 2% clear solution of chitosan. AgNPs-NWCF was soaked in 2% chitosan solution for 2 minutes. In a similar way, second coating was done in fresh chitosan solution of the same concentration. Between each coating, fabric samples were washed with distilled water subsequently to remove unbound and loosely attached chitosan and were dried. Chitosan modified fabric samples were then padded through a laboratory wringer at low-pressure settings, oven-dried at 60 °C for 60 minutes. All the samples obtained were kept at 105 °C for 4 minutes, dried, and stored for further analysis. The samples prepared were termed as chitosan@ AgNPs-NWCF. The control fabrics were prepared with the same conditions without the addition of chitosan.

### ***N-halamines* Formation on Chitosan@ AgNPs-Nonwoven Cotton Fabric**

*N-halamine* functionalities were created at the surface of chitosan modified AgNPs-nonwoven cotton fabric to obtain oxidizing halogen moiety i.e chlorine, as described in earlier reports with slight modifications.(Cheng, Ma et al. 2014) It is generally believed that amine groups in chitosan having N-H bond as precursor changed into N-X bond form *N-halamine* at the surface of the fabric. In the present study, modified cotton fabric was dipped into 10 mL of 10% household bleach (sodium hypochlorite solution 6% Cl<sup>+</sup>) solution at 7pH for 30 minutes at room temperature. After chlorination, the fabric was rinsed with deionized water several times to remove any unbound and free chlorine from the fabric surface. The fabric was then dried at room temperature for 24 hours. The modified fabric was taken for further analysis and characterization. The samples prepared were termed as *N-halamine*-chitosan@ AgNPs-NWCF. The control fabrics were prepared with the same conditions without treating with sodium hypochlorite solution.

### **Analysis of Loaded Chlorine Contents on Chitosan@ AgNPs-Nonwoven Cotton Fabric (NWCF)**

Loaded chlorine contents on all prepared samples were measured by using the iodine/ thiosulphate titration procedure (Worley, Chen et al. 2005). Generally, 1g of KI was added to 40mL of CH<sub>3</sub>COOH (1%) containing 0.06g (0.85 cm ×1.0cm) of *N-halamine*-chitosan@ AgNPs NWCF, solution color does not change. Solution was stirred vigorously for one hour, color changed slowly from transparent to light yellow. After one hour of stirring, KI solution containing *N-halamine*-chitosan@ AgNPs NWCF was titrated

with 0.01M sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), until the endpoint (colorless). For comparison, the whole procedure was also performed for chitosan@ AgNPs NWCF and AgNPs NWCF without treating with chlorine. The final volume of sodium thiosulfate consumed during titration was calculated. The loaded chlorine concentration for modified and unmodified fabric samples was calculated by using the following equation (Eq.1).

$$Cl (\%) = \frac{35.5}{2} \times \frac{(V_{cl} - V_0) \times 10^{-3} \times 0.01}{W_{cl}} \times 100 \dots \dots Eq. 1$$

Where 35.5 amu is the atomic mass of loaded chlorine.  $W_{Cl}$  weight (g) of the fabric piece,  $V_0$  and  $V_{Cl}$  are volumes of  $\text{N}_2\text{S}_2\text{O}_3$  solution consumed during titration of chlorine-treated and untreated nonwoven cotton fabric, respectively.

### Photostability and Shelf-life of *N-halamine*-Chitosan@AgNPs-Nonwoven Cotton Fabric

The photosensitivity and shelf life of fabric samples, chitosan@ AgNPs-NWCF, chitosan@ AgNPs-NWCF and *N-halamine*-chitosan @ AgNPs-NWCF with dimensions of 0.85 cm ×1.0 cm were exposed to a UVA lamp (345nm) for 6 weeks at room conditions. The samples were investigated at different time intervals of 2, 4, and 6 weeks for loss of chlorine contents. Similarly, in another experiment samples were stored in dark under the same experimental conditions. The stability in terms of loss of chlorine contents and durability for all samples was measured using iodine/ thiosulphate titration. The percent loss in chlorine loading for all samples under light and dark was calculated and compared..

## Antimicrobial Efficacy Analysis

Two types of tests were conducted to determine the antibacterial efficacy of modified non-woven cotton fabric samples as described earlier. (Grabchev, Staneva et al. 2019; Gao, Su et al. 2020) *Micrococcus lutes*, *Staphylococcus aurea* (AATCC (Test Method 100 of American Association of Textile Chemist and Colorists 6538) were used as a gram-positive bacterium, and *Enterobacter aerogenes*, *Escherichia coli* (O157: H7, AATCC 43895) were used as a gram-negative bacterium. Generally, (0.85 cm ×1.0cm dimensions) AgNPs- nonwoven cotton fabric (Control-I) was taken for analysis as described. Firstly, the bacterial suspension was prepared in phosphate buffer (pH7) to generate a known population (Colony Forming Units, CFU). 25 mL of the bacterial suspensions containing  $1.7 \times 10^5$  CFUS buffered at pH7 was added to the center of AgNPs-nonwoven cotton fabric (Control-I). Bacterial colony was covered with second AgNPs- nonwoven cotton fabric (Control-I) of the same composition and dimension. After 5 minutes, the fabric piece was taken off, quenched with 5.0 mL of sterile 0.02N sodium thiosulfate solution to remove and neutralize all oxidative chlorine. The samples were vortexed for 3 min to remove all bacteria from fabric surfaces. Bacteria-containing aliquots were diluted with 100 mL PBS (pH7). Aliquots were transferred to 6mm Trypticase soy agar disk plates, incubated at 37 °C for 24 h, and counted for viable CFU of bacteria. The same procedure was followed for the other two fabric pieces of

(0.85 cm × 1.0 cm dimension) chitosan@AgNPs nonwoven cotton fabric (termed as Control-II) and *N-halamine*-chitosan@AgNPs nonwoven cotton fabric (Sample). The bacterial inhibition growth analyses were carried out in triplicate.

## Cell viability Studies Using MTT Assay

MTT assay is done to evaluate the cell viability of all nonwoven modified fabric samples in Hella cell lines culture following reported procedures. (Fang and Trewyn 2012; Grabchev, Staneva et al. 2019; Gao, Su et al. 2020) The cells were grown as monolayer cultures in a DMEM medium. The cultures were kept at 37 °C in a humidified incubator (5% CO<sub>2</sub>, 95% air) . All experiments were performed during the exponential phase of cell growth. The cells were grown in 96-well microplates for cell culturing at a concentration of 1 × 10<sup>4</sup> cells/ well for 24 hrs. The culture medium was then removed and transferred with modified and unmodified nonwoven fabric. Samples of the cells viability in the presence of AgNPs-NWCF and chitosan@AgNPs-NWCF served as the negative control. The cell viability studies of the AgNPs-NWCF, chitosan@AgNPs-NWCF and N-halamine chitosan@AgNPs-NWCF against *HeLa cell* lines was evaluated using a colorimetric method in accordance with Mossman's procedure. The cells were incubated for 3 h with 5mg MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) 10 mL DMEM solution ) at 37°C (5% carbon dioxide) in FBS free medium. The formed blue MTT formazan was extracted with a mixture of absolute ethanol and DMSO (1:1). The quantitative analysis was performed by absorbance measurements in an automatic microplate reader at 460 nm. The total cell viability is calculated by following equation and expressed as percentage of control.

$$\text{Cell viability} = \frac{(A_{NWCF} - A_{blank})}{(A_{control} - A_{blank})} \times 100 \% \dots \dots \text{Eq. 2}$$

Where, A = absorbance measures at 460 nm.  $A_{NWCF}$  for modified and unmodified fabric samples,  $A_{control}$  for positive control and negative control. The values were calculated for triplicate measurements.

## Results And Discussion

### Synthesis and Characterization of Modified Nonwoven Fabric Samples

Samples of fabrics were modified following three step process, cleaning and activation of fabric surface, coating with silver nanaoparticles and N-halamine formation through chlorination of chitosan at fabric. The modification was carried out simply by using dip coating method. This method of coating is considered as a facile, environment friendly, requires fewer chemicals, and is cost effective. (Ceratti, Louis et al. 2015) Dip coating method is widely used in many industrial and laboratory scales methods for the coating of surface of different substrates. (Tang and Yan 2017) All experiments were carried out at room temperature. The successful attachment of modifiers on non-woven cotton fabric was investigated further using different spectroanalytical techniques. Surface Characterization of nonwoven cotton fabric

modified with AgNPs, chitosan@AgNPs, and *N-halamine*-chitosan@AgNPs was performed by FTIR (Fourier Transform Infrared) spectroscopy, SEM, and XRD. FTIR spectra (Figure 1,a) shows characteristic peaks for AgNPs-nonwoven cotton fabric at  $3348\text{ cm}^{-1}$  for O-H bonds (stretching vibration), at  $2910\text{ cm}^{-1}$  for C-H bond (stretching vibration) and at  $1313\text{ cm}^{-1}$  (C-H bending vibration). Peak due to the C-O bond is intense and broad, shifts to  $1053\text{ cm}^{-1}$  because of AgNPs coated on nonwoven cotton fabric. These results reveal the bonding of silver nanoparticles with O-atoms of cellulose of the fabric. Coating of chitosan on the surface of AgNPs-nonwoven cotton fabric was also characterized by FTIR. Peaks at  $3278\text{ cm}^{-1}$  and  $2890\text{ cm}^{-1}$  belong to -OH, -NH<sub>2</sub>, and aliphatic groups and absorption peaks at  $1557\text{ cm}^{-1}$  depict the N-H bending vibrations. Broad peak (at  $3400$  to  $3500\text{ cm}^{-1}$ ) confirm the existence of the amine group in chitosan which is also overlapped with the broad peak of O-H. C-H bending vibration absorption at  $1313\text{ cm}^{-1}$  and C-O in cellulose has an intense peak at  $1053\text{ cm}^{-1}$  show the broad and red-shifted due to the bonding of AgNPs and oxygen atoms as shown in Figure 1(b). There is bond formation as N-X by halogenation of N-H groups. O-H bond (stretching vibration) in chitosan and cellulose molecules show absorption peaks at  $3254\text{ cm}^{-1}$ , C-H bond (stretching vibration) occurs at  $2913\text{ cm}^{-1}$ , and (C-H bending vibration) absorption peak occurs at  $1388\text{ cm}^{-1}$ . Stretching vibration of carbon and oxygen bond occurs at  $1010\text{ cm}^{-1}$ . Amine group in chitosan has a particular absorption peak in the region from  $3400$  to  $3500\text{ cm}^{-1}$ . Absorption peaks at  $1,557\text{ cm}^{-1}$  decreased in intensity because after chlorination N-H bond converts into N-Cl bond or the formation of *N-halamine* occurs. FTIR absorption spectrum of *N-halamine*-chitosan@AgNPs-nonwoven cotton fabric is shown below in Figure 1(c).

AgNPs, chitosan, and *N-halamines* modified fabric surface was further characterized by SEM. SEM image of nonwoven cotton fabric is shown below in Figure 2 (a). AgNPs modified nonwoven cotton fabric show morphological changes. Some physical changes after the adsorption of silver nanoparticles on the surface of the fabric were also analyzed with SEM. It demonstrates that AgNPs coated NWFC has a smooth surface and uniform distribution of particles. SEM image of AgNPs-nonwoven cotton fabric is shown in Figure 2 (b). SEM photograph of chitosan coating on AgNPs-nonwoven cotton fabric is clear. Crystallites are uniformly distributed. Accumulation of small particles can also be observed. SEM image of chitosan/ AgNPs-nonwoven cotton fabric is shown in Figure 2(c). Loaded chlorine slightly alters the structure of chitosan coating. Aggregation of tiny particles can also be observed. SEM image of *N-halamine*-chitosan@AgNPs-nonwoven cotton fabric is shown-Figure 2 (d).

XRD analysis of modified and unmodified nonwoven cotton fabric was carried out to observe the structural changes. X-ray diffractometer was used for XRD analysis. Measurements were taken at 40 kV and 40 mA in  $2\theta$  range  $10$  to  $80^\circ$  with Cu-K $\alpha$  radiation ( $\lambda = 0.15418\text{ nm}$ ,  $0.05$  degrees *step size*,  $1\text{ sec per step}$ ) (Ling, Wang et al. 2019; French 2020). XRD spectra of non-woven cotton fabric are shown in (Figure 3). The peaks at  $38.6^\circ$ ,  $44.9^\circ$ ,  $64.4^\circ$ , and  $77.9^\circ$  obtained correspond to AgNPs-NWCF type represent the presence of silver in the fabric. (Figure 3 a) These peaks belongs to silver nanoparticles are similar as reported earlier. (Jyoti and Singh 2016). Figure 3(b) for chitosan coating on AgNPs-NWCF shows characteristic peaks at  $16^\circ$  and  $22^\circ$  reveal chitosan with crystalline (a hydrated) structure. (Jampafuang,

Tongta et al. 2019). High-intensity peak at  $22^\circ$  represent the face-centered cubic material. X-ray diffraction pattern of chitosan@AgNPs- NWCF after formation of *N*-halamine shows most prominent peaks at  $22^\circ$ ,  $39^\circ$  and  $46^\circ$  with small less intense peaks at more diffraction angles. Figure 3(c) The small peaks represent the small size nanoparticles. (Sun and Sun 2004). All peaks are broad as compared to AgNPs-NWCF and chitosan@AgNPs-NWCF samples. It revealed that after chlorination of chitosan a new layer of chlorine atoms deposited and changes the diffraction angles. XRD pattern in addition also elucidated the nonwoven sample with and without modifiers are impurities free, surface remains the same and no additional chemical reaction takes place after coatings.

### **Preparation and Characterization of Antimicrobial Coating on Nonwoven Cotton Fabric**

In industries, during fabric preparation, different types of chemicals or reagents are applied which protect it from insects, microbes, and other fungus attacks. Fabric surface is cleaned from residual chemicals before the surface modification process. In present study nonwoven fabric surfaces are modified using dip-coating approach. The approach is simple, eco-friendly, cost-effective, and easy to execute. Surface roughness on the nonwoven cotton fabric is achieved with a 20 mL 6M potassium hydroxide after cleaning with detergents and distilled water several times. Higher concentration of alkaline solution generated a higher number of active sites for further modification. Surface hydroxyl groups (OH) of the fabric cellulosic surface generates cellulose-OK<sup>+</sup> on hydrolysis with potassium hydroxide. Cleaned and negatively charged surfaces of the fibers are further modified with in situ synthesis of silver nanoparticles. Alkali-treated cellulose-OK<sup>+</sup> nonwoven fabric is dipped in AgNO<sub>3</sub> solution. Reactive sites on the alkali-treated fabric are exchanged with Ag ions creating cellulose-OAg<sup>+</sup>. Silver ion modified surface is reduced further with ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>) aqueous solution to generate silver nanoparticles (AgNPs) on the surface. Change in fabric color from white to dark brown confirms the presence and formation of silver nanoparticles at fabric. Mechanism and schematic illustrations is shown in **Figure 4**. *N*-halamine is the derivatization of chitosan polymeric materials with haloamine functional groups. This modification has attracted much attention in recent years due to its quick antibacterial response and vast potential in other biomedical and food packaging applications. Chitosan-AGNPs surfaces are positively charged that form covalent bonds with negatively charged chlorine from household bleach, requiring no additional surface modification. Therefore, chitosan-AgNPs surface is modified with an *N*-halamine precursor simply by dipping.

### **Loaded Chlorine Content Analysis**

Loaded chlorine content on AgNPs- NWCF, chitosan@ AgNPs- NWCF and *N*-halamines-chitosan@ AgNPs- NWCF is determined with simple iodine/ thiosulphate titration procedure. 0.06 g (0.85 cm × 1.0 cm) of *N*-halamines-chitosan@ AgNPs-NWCF (for 30 minutes consumed 1mL sodium thiosulphate standard solution during titration. AgNPs- NWCF (Control-I), chitosan@ AgNPs- NWCF (control-II) of same weight and dimensions consumed 0 mL sodium thiosulphate standard solution. Sodium thiosulphate volume is used to calculate the loaded chlorine content on chlorinated and unchlorinated fabric, respectively as reported in earlier reports.(Liang, Chen et al. 2007; Cheng, Ma et al. 2014; Demir, Cerkez et al. 2015) *N*-

*halamines*-chitosan@ AgNPs-NWCF contain 0.295% and AgNPs- NWCF, chitosan@ AgNPs- NWCF contain 0% loaded chlorine concentration. The obtained results are shown in **Table S1** (supplementary information).

### **Antibacterial Efficacy Testing for *N-halamine*-chitosan@ AgNPs-NWCF**

Biocidal properties of AgNPs- NWCF, chitosan@ AgNPs- NWCF, and *N-halamines*-chitosan@ AgNPs-NWCF samples tested against four types of bacteria as *Micrococcus lutes* (Gram-positive), *Staphylococcus aurea* (Gram-positive), *Enterobacter aerogenes* (Gram-negative), and *E.coli* (Gram-negative) following established procedures (Cheng, Ma et al. 2014) as detailed in material and methods section and schematically in **Figure (5a)**. The fabric samples were exposed to bacterial strains at  $1.7 \times 10^5$  CFU / fabric patch. Figure 5b response graph and **Table S2**(supplementary information) show the antibacterial results. The samples of AgNPs-NWCF and chitosan@AgNPs-NWCF chitosan offered low bactericidal activity against *Micrococcus lutes*, *Staphylococcus aurea*, *Enterobacter aerogenes*, and *E.coli* strains. The log reductions of 6.7, 6.6, 5.6, 5.4 and 7.8, 7.7, 7.5, 7.3, respectively were observed for aforementioned bacterial strains. The modified fabric samples provided about 11.2 to 11.9 log reduction within 15 min of exposure time compared to control. The inhibition efficacies of the *N-halamine*-chitosan@ AgNPs-NWCF improved significantly compared with other samples. The reduction in number of bacteria in media is attributed to the attachment of bacteria to the fabric samples and the inactivation by the *N-Halamine* and silver nanoparticles modified fabric. It is assumed that the inactivation of bacterial growth has been achieved through transfer of positive halogen ( $Cl^+$ ) from *N-halamine* coating to the growth medium. In addition *N-halamine* containing stable N-X bond as shown in XRD and FTIR pattern also tends to add killing effect through contact with fabric surface.(Ahmed, Hay et al. 2009; Ren, Akdag et al. 2009; Bai, Kang et al. 2018; Wang, Huang et al. 2020) More interestingly, it is proposed that the antibacterial activity cannot be explained alone due to halogen release or contact but also combination effect of silver nanoparticles and *N-halamine* simultaneously. The results are similar to previous reports (Bai, Zhang et al. 2016) The *N-halamine*-chitosan@ AgNPs-NWCF samples inhibits bacterial growth, with Gram-negative bacteria having lower bacterial activity than Gram-positive bacteria. This fact is attributed to the different shapes, size, surface structures and more resistance to inactivation of bacteria over other strain. (Cheng, Ma et al. 2014) The results are equal to or consistent with the previously reports. (Liang, Chen et al. 2007; Li, Hu et al. 2013; Demir, Cerkez et al. 2015; Chylińska and Kaczmarek 2020)

### **Cytotoxicity studies of *N-halamine*-chitosan@AgNPs-NWCF**

The effect of modifiers N-halamine, chitosan and silver nanoparticles on cell survivability on the *Helacells* was evaluated. (Figure 6) The cell viability analyses tell about the number of living cells after treatment as percentage against positive control. In the present study, cell viability % was calculated with respect to AgNPs-NWCF, chitosan@AgNPs-NWCF, *N-halamine*-chitosan@AgNPs-NWFC samples against sodium lauryl sulfate solution (2%) as positive control *N-halamine*-treated Chitosan@AgNPs-NWFC samples showed cell viability by up to 85%. These results suggested that N-halamine modified nonwoven cotton

fabric not significantly inhibit the cell viability. The deviation in percentage from 100 % value may attributed to the  $\text{Cl}^+$  ions released from fabric surface and might have interfere with the cell viability measurements. Compared to *N-halamine*-Chitosan@AgNPs-NWFC, AgNPs-NWCF, Chitosan@AgNPs-NWCF showed 25% and 30% cell viability, respectively, which shows their significant toxicity towards *HeLa cell* Lines. These results suggest that *N-halamine* modification do not significantly possess cell toxicity; the results are in agreement with other reported results for similar studies. (Demir, Cerkez et al. 2015; Demir, Broughton et al. 2017; Grabchev, Staneva et al. 2019; Gao, Su et al. 2020) However, it is also assumed that the small toxicity observed in the present experiment may due to release or dissociation of  $\text{Cl}^+$  into cell medium with time from *N-halamine* modified chitosan@AgNPs-NWFC. The release can be controlled by optimizing the chlorine contents at nonwoven cotton fabric and with repeated washing with water without affecting fabricated fabric properties.

## Photostability and Shelf Life Stability

Nonwoven cotton fabric samples are investigated for Photostability and shelf life at room temperature. It has been reported in the literature that N-Cl bond in N- halamine shows sensitivity towards light irradiations. The dissociation of bonds increased with exposure to UVA light. (Ma, Li et al. 2019). The shelf life (storage) stability of bound chlorine in *N-halamines*-chitosan@AgNPs-nonwoven fabric under UVA lamp and dark environment for 12 weeks are shown in **Figure S1** ( Supplementary Information). *N-halamines*-chitosan@AgNPs-NWCF stored in dark environmental conditions retained most of their initial chlorine loadings for 6 weeks. *N-halamines*-chitosan@AgNPs-NWCF lost only 20% of chlorine after 12 weeks of storage in darkness. It is observed that *N-halamines*-chitosan@AgNPs-NWCF excellent storage stability and retained  $80\% \pm 10$  of their chlorine content after 12 weeks in a dark environment. However, *N-halamines*-chitosan@AgNPs-NWCF is somewhat less stable under the light. It is noted that the N-Cl bond dissociation increased with exposure to UVA light. When stored under light, *N-halamines*-chitosan@AgNPs-NWCF samples lost only 70% of the oxidative chlorine over 12 weeks of storage. Photostability of *N-halamine* modified data is comparable with previous reports. (Chylińska and Kaczmarek 2020) It can be concluded that the present modification method is stable, simple, low cost and environment friendly yet to be established in laboratories for various biomedical applications.

## Conclusion

A cost-effective and ecofriendly nonwoven cotton fabric with antibacterial and nontoxic properties is fabricated in this study. N-halamine and silver nanaoparticles have been coated onto nonwoven cotton fabric sample using dip coating method. The FTIR, SEM and XRD analysis were used to characterize the modified surface. The analysis showed successful coating and functionalization onto nonwoven cotton fabric, and not damaged under the acidic conditions of treatment. The antibacterial test showed a significant decrease in the bacterial colony on exposure to *N-halamines*- chitosan @AgNPs-nonwoven cotton fabric as compared to AgNPs-nonwoven cotton fabric, chitosan@AgNPs-nonwoven cotton fabric. Addition of *N-halamines* onto samples increases antibacterial activity of gram positive bacteria than

gram negative bacteria. Moreover, the *N-halamines* modified chitosan @AgNPs-nonwoven cotton fabric showed no apparent cell toxicity against HeLa cell lines. These encouraging results indicate that *N-halamine*-chitosan @AgNPs-nonwoven cotton fabric may have a wide range of applications in biomedicine and health care, including wound dressings, surgical masks, coatings for medical devices, and hospital linens.

## Declarations

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**Authors Contribution:** Conceptualization, methodology, fabric materials treatment, original draft preparation, R.N, N. Z. and S.T.R.N ; review, editing, graphics, S.T.R.N, B.F, M.U.F, D.H, S.M .; characterization, W.Q.K, S.M.; visualization, cell viability studies, antibacterial studies, R.N, N.Z, A.R,M.U.F, A.J, L.J ; supervision, S.M.

### Conflict of interest

The authors describe no conflict of interest in this work.

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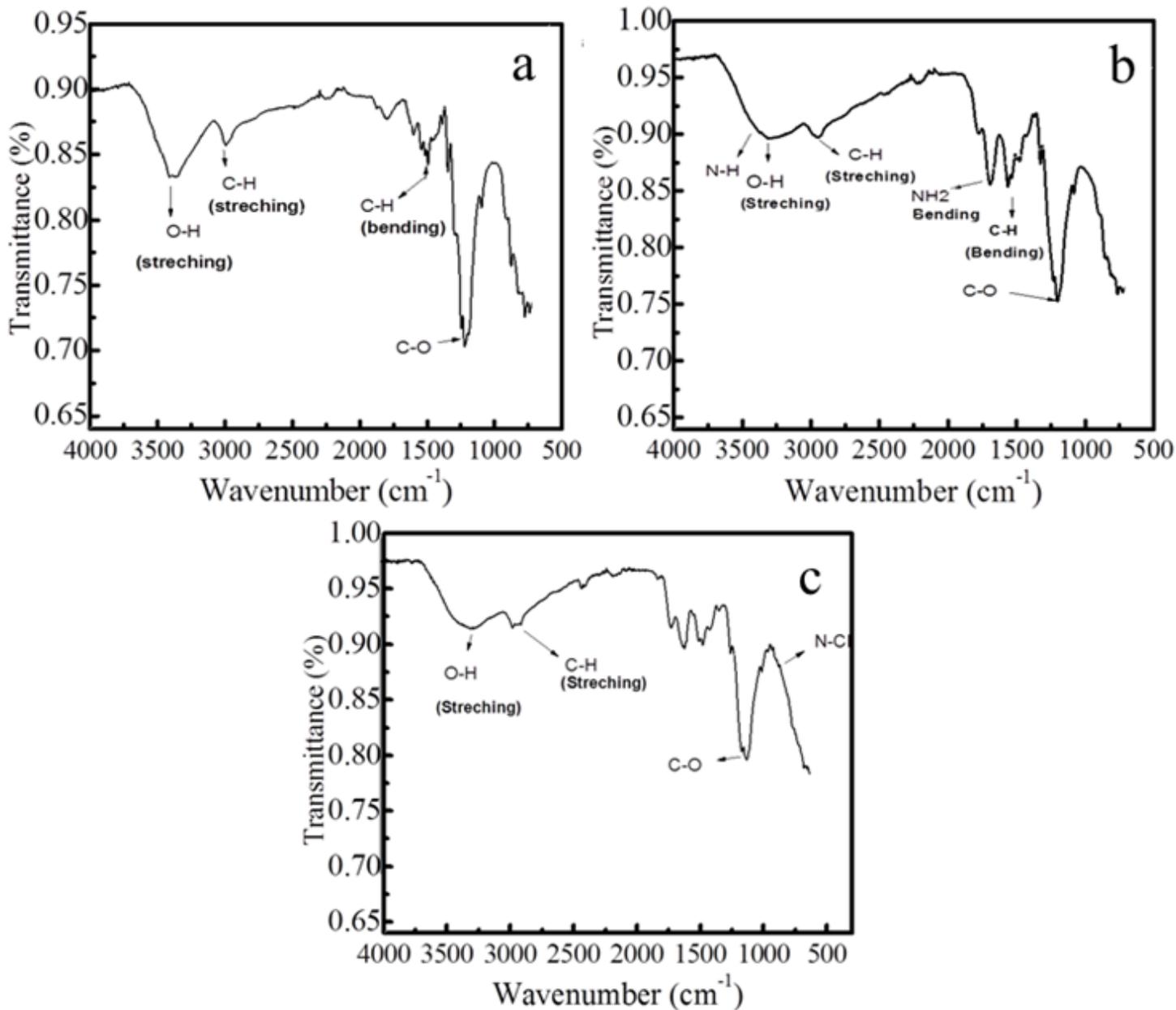
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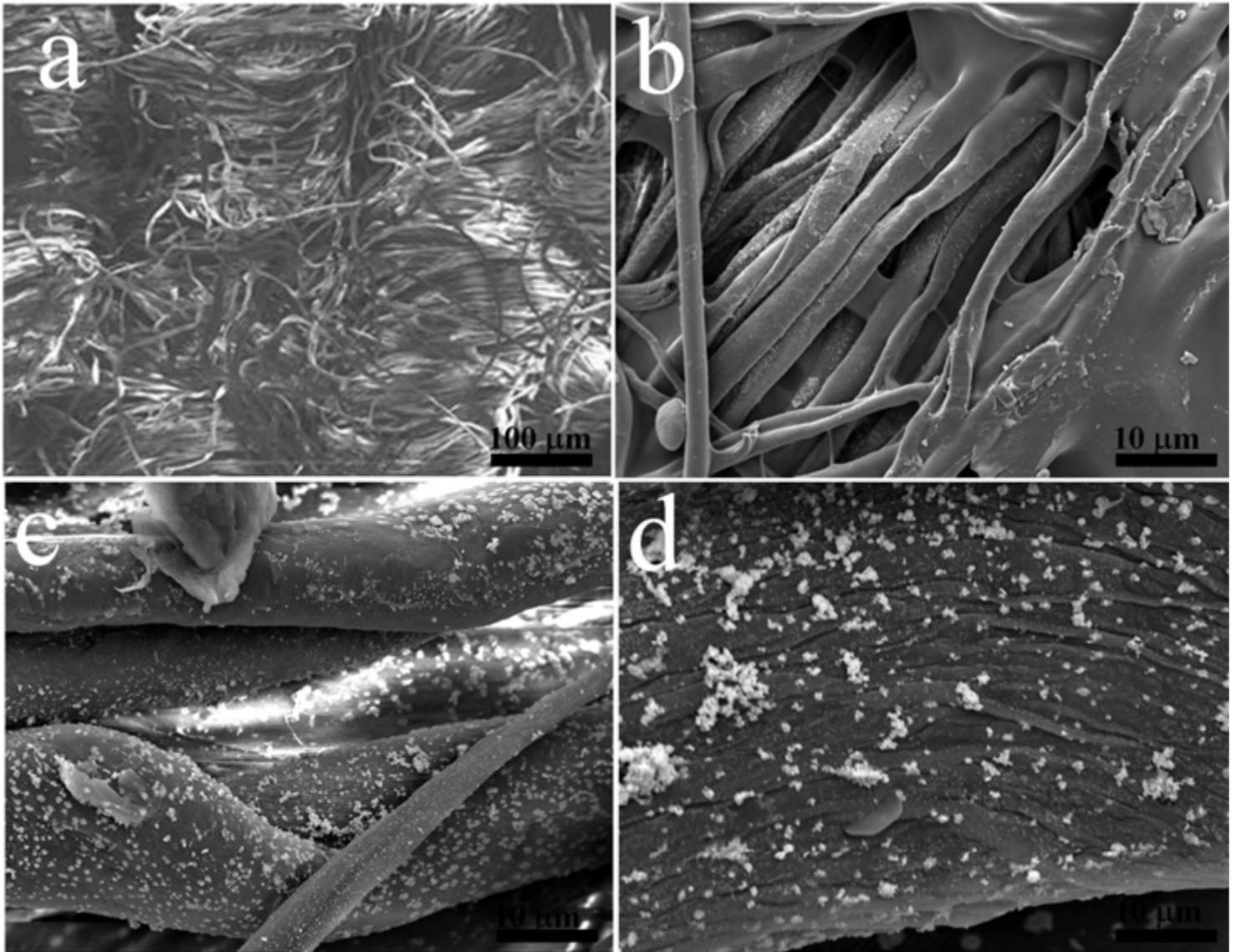
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## Figures



**Figure 1**

FTIR spectra of AgNPs-nonwoven cotton fabric (a), Chitosan@AgNPs nonwoven cotton fabric (b), and N-halamine-chitosan@AgNPs-nonwoven cotton fabric (c).



**Figure 2**

SEM. images of non-woven cotton fabric (NWCF) (a) AgNPs-NWCF (b) Chitosan@AgNPs-NWCF(c) N-halamine-chitosan@AgNPs-NWCF (d).

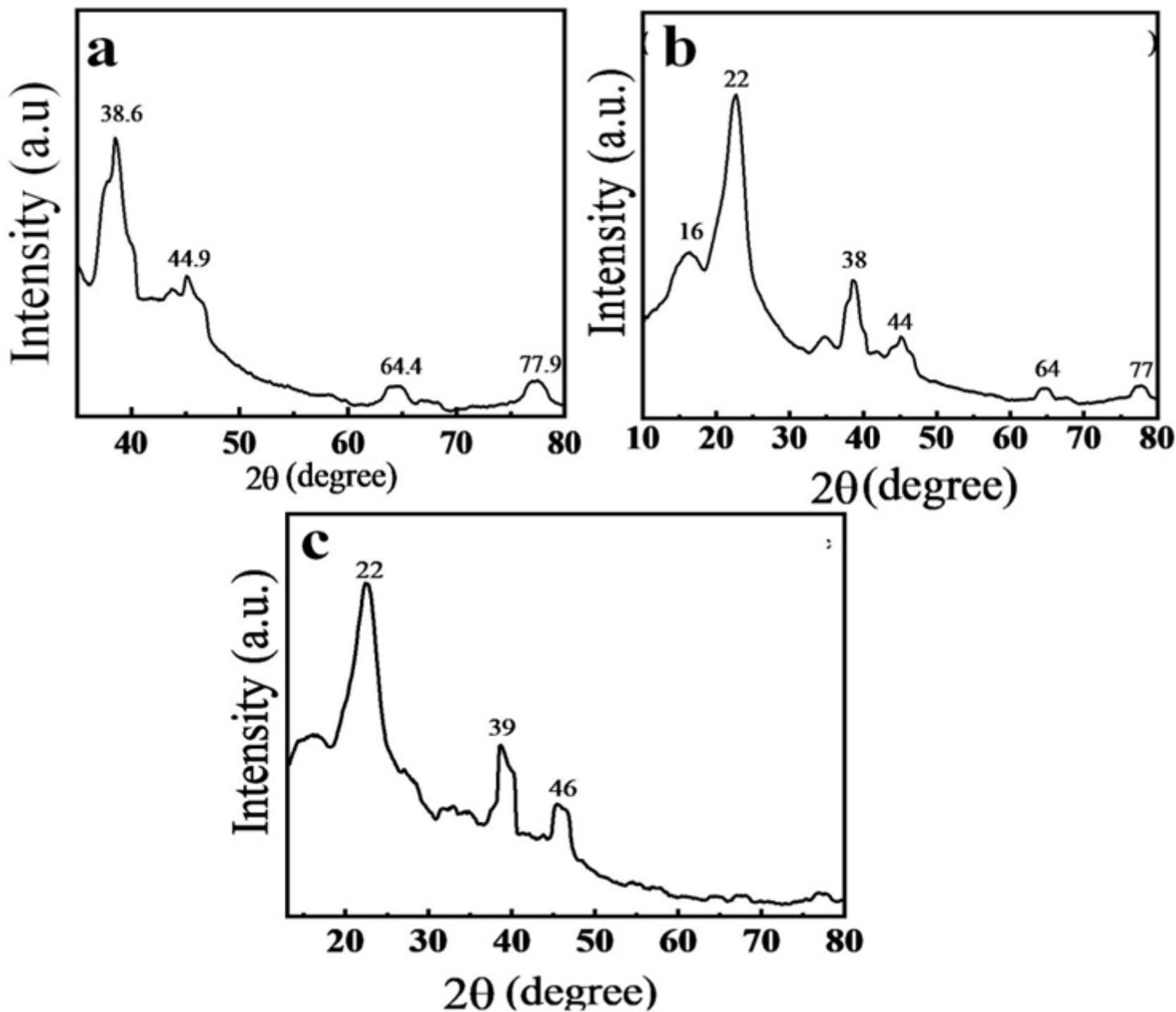
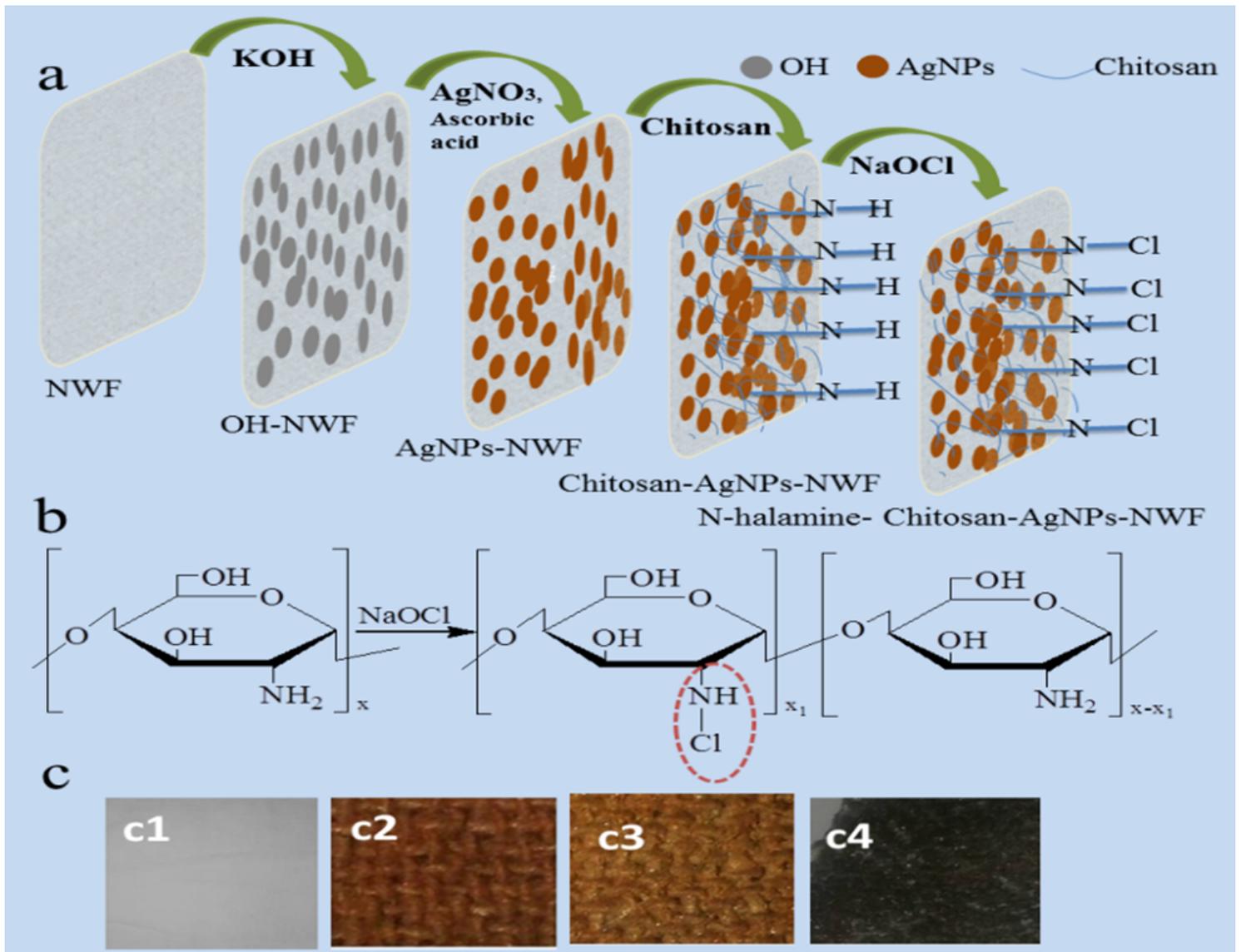


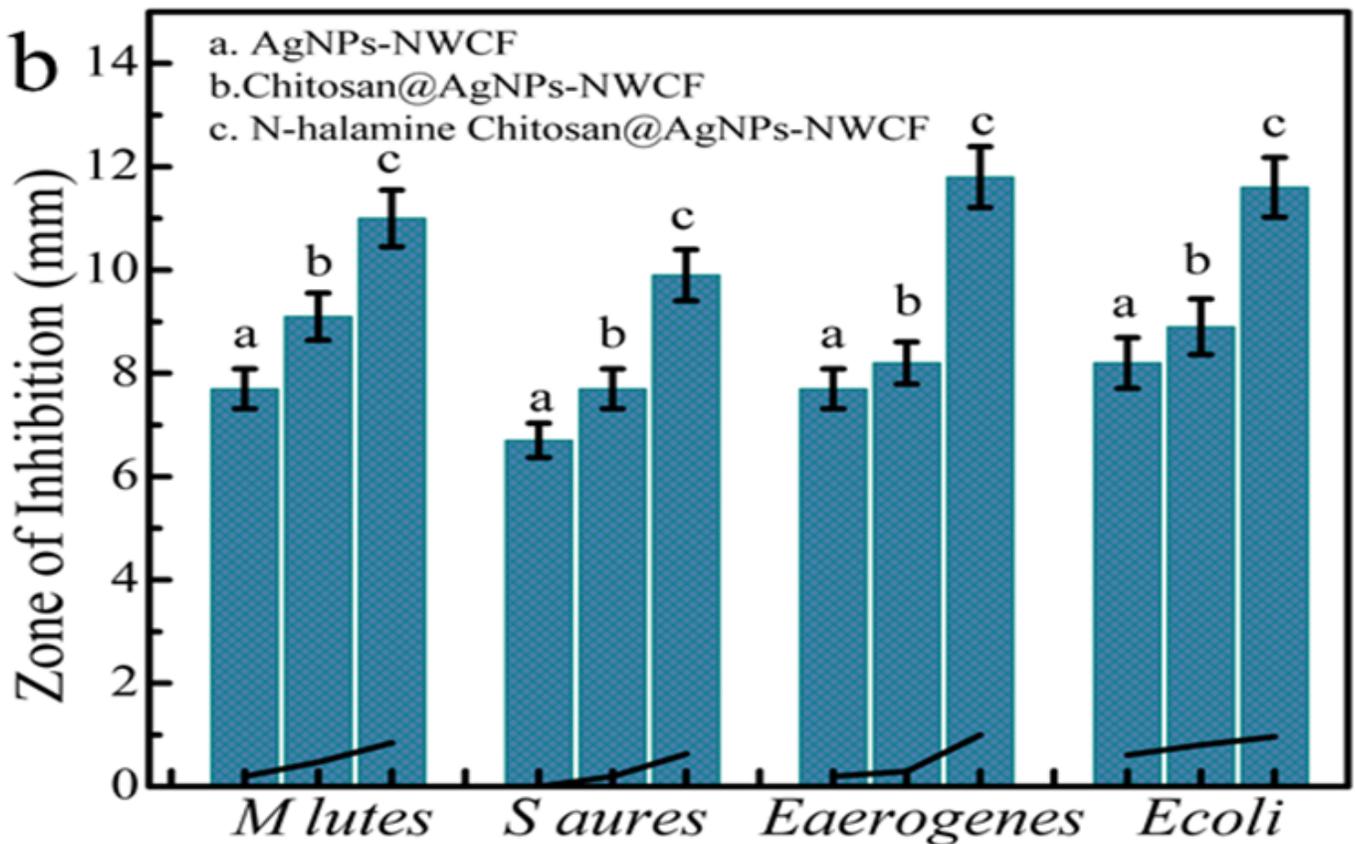
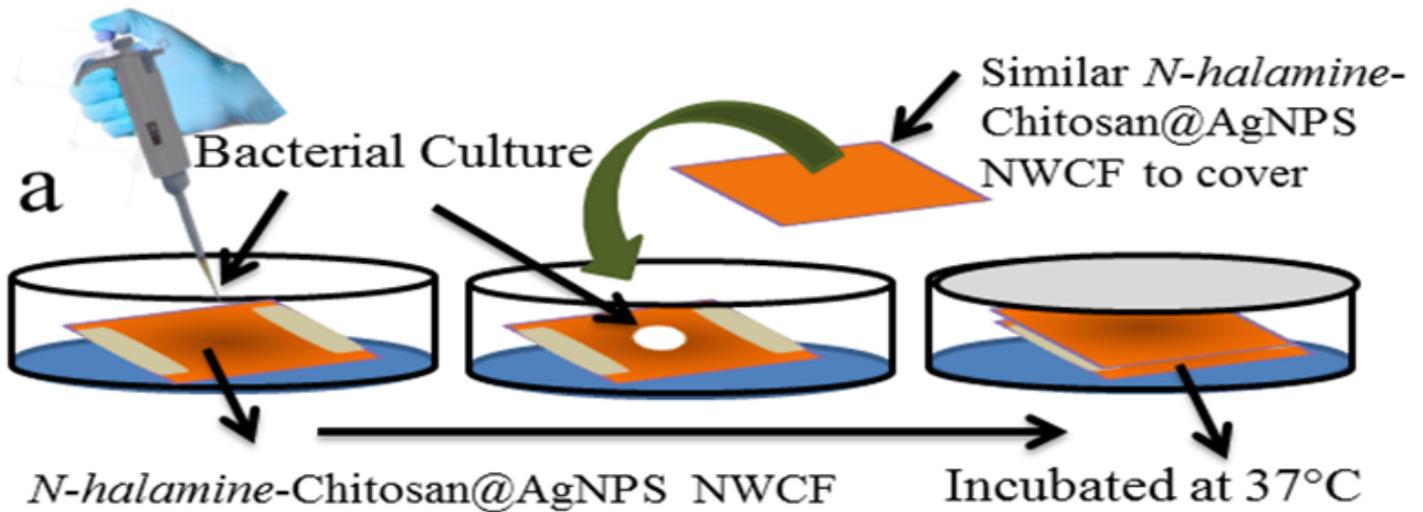
Figure 3

XRD patterns of AgNPs-nonwoven cotton fabric (a), Chitosan@AgNPs nonwoven cotton fabric (b), and N-halamine-chitosan@AgNPs-nonwoven cotton fabric (c).



**Figure 4**

Modification and coating steps of nonwoven cotton fabric (a), Schematic illustration for halogenations of Chitosan (b) at fabric surface and Visual color changes on fabric after coating and modification steps (c) non-woven cotton fabric (c1), the dark brown color obtained on modification with AgNPs (c2), the brown color obtained on chitosan coating (c3) and after N-halamine formation(c4).



**Figure 5**

Schematic illustration (a) for measurement of antimicrobial efficacy of nonwoven cotton fabric, the response of growth inhibition zone (b) of AgNPs-NWCF (Control-I), chitosan@AgNPs-NWCF (Control-II), and N-halamines-chitosan@AgNPs-NWCF (Sample) for four types of bacteria. An error bar has been added for n=3. The trend line at bars shows the response behavior of bacteria for control (I, II) and modified sample.

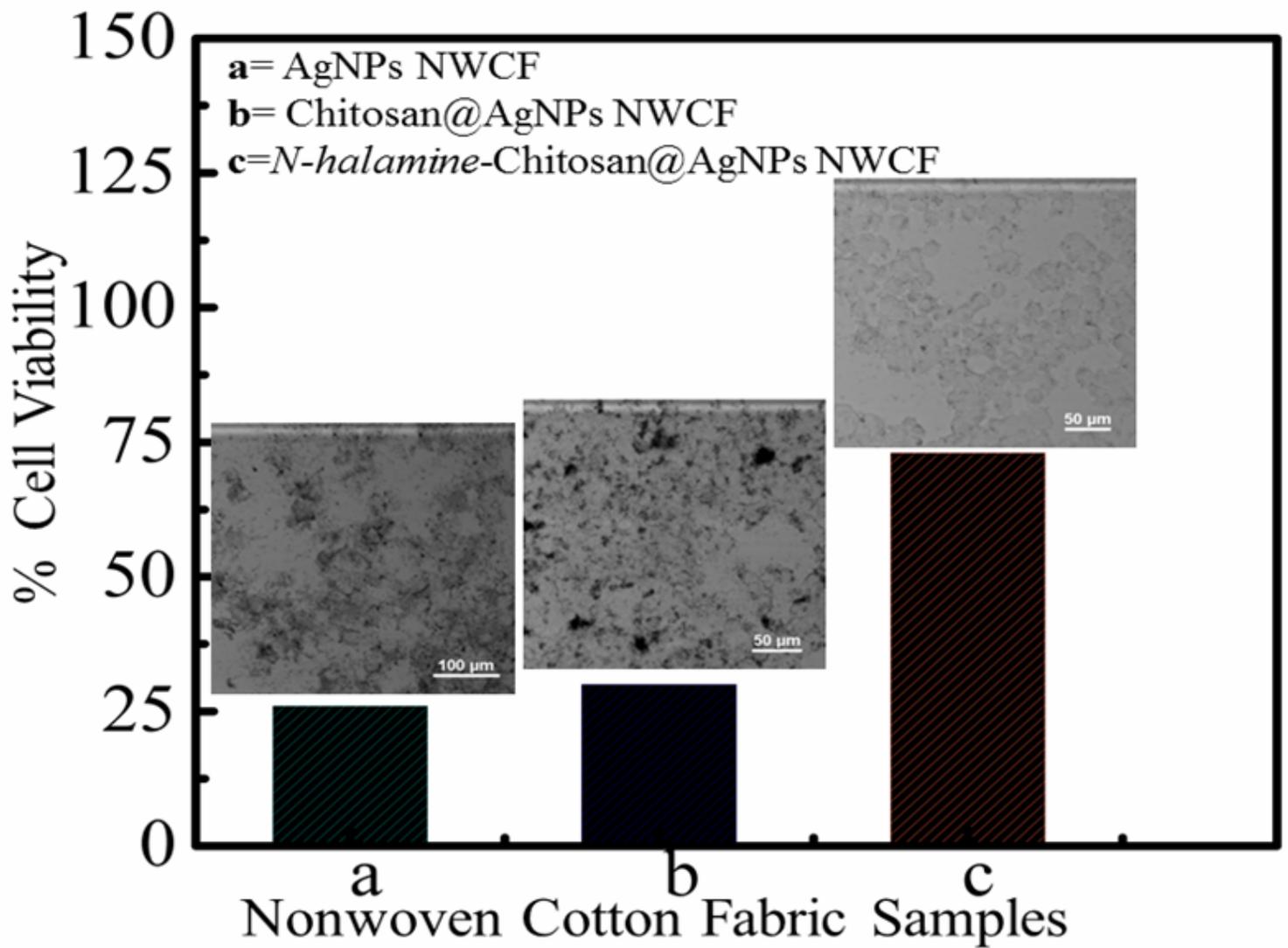


Figure 6

Cell viability analysis (MTT assay) on Hela Cell lines of AgNPs-NWCF, Chitosan@AgNPs-NWCF, N-halamine-Chitosan@AgNPs-NWCF. Inside images show confocal microscopic cell images.

## Supplementary Files

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