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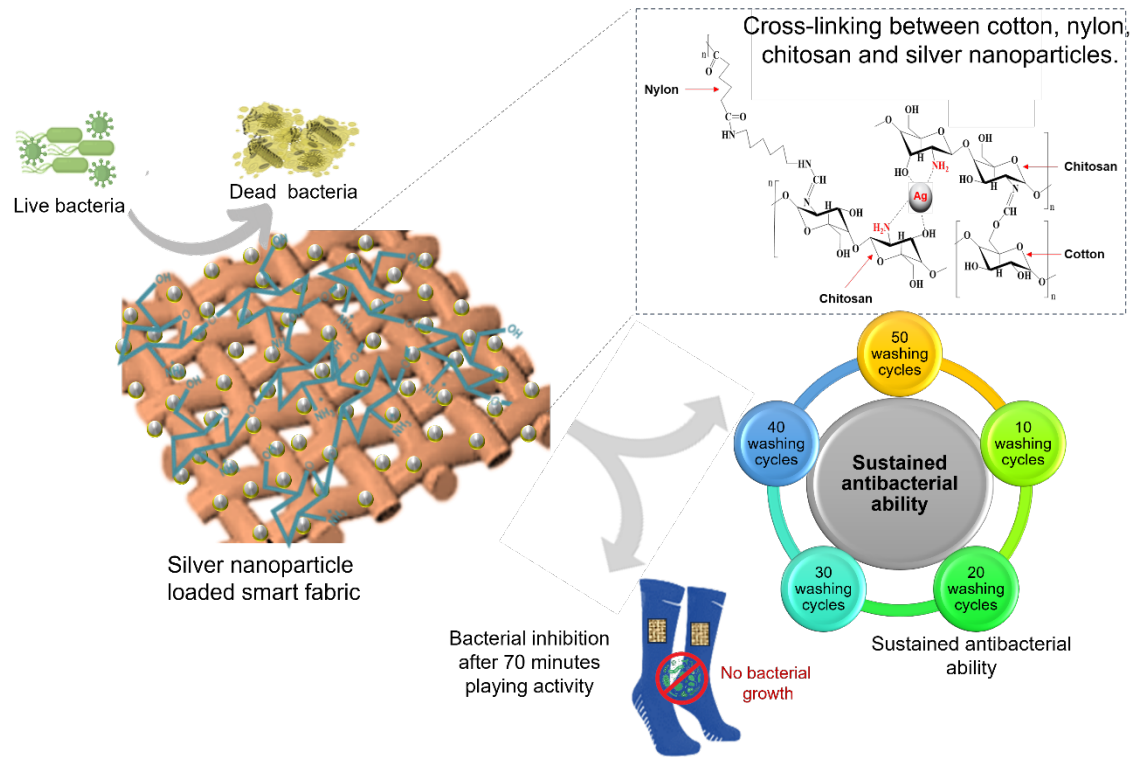
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Graphical abstract



A novel methodology for stabilization of silver nanoparticles on cotton, nylon, and cotton/nylon fabrics using chitosan and triethyl orthoformate for enhanced and elongated anti-bacterial performance

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Abstract

In the present study, the commercially available cotton, nylon and cotton/nylon fabrics were modified by chitosan and silver nanoparticles using a crosslinker triethyl orthoformate (TEOF). Resulted cotton-silver (Ag-Cs-Cot), nylon-silver (Ag-Cs-Nylon) and cotton-nylon silver (Ag-Cs-Cot-Nyl) fabrics showed significant anti-bacterial activity even after 50 washing cycles. Silver nanoparticles were prepared by reducing silver nitrate through sodium borohydride at 0°C. In FTIR spectra, the peak at 1650 cm⁻¹ confirmed that TEOF mediated attachment of chitosan with fabrics and the stretch of secondary amine near the 3375 cm⁻¹ indicated the silver attachment to the amine group of the chitosan. In scanning electron microscopy (SEM) images smooth surfaces of fabrics without any damage by modification process were observed. The antibacterial activity was analysed by agar diffusion and broth dilution assays against *Escherichia coli* and *Staphylococcus aureus* bacterial strains and results showed 90% bacterial inhibition against *E. coli* and 89% bacterial inhibition against *S. aureus*. For testing the antibacterial durability, the modified fabrics

were washed with non-ionic detergent (10g/l) for 15 minutes under aggressive stirring (100RMP) at room temperature. The modified fabrics retained anti-bacterial activity over the 50 washing cycles. Finally, the commercial potential of cotton-silver fabric was evaluated by stitching it with the socks of football players and interestingly results showed that the modified fabric on the socks showed more than 90% bacterial inhibition as compared to the plain fabric after 70 minutes of playing activity.

1. Introduction

Bacterial attachment and the subsequent growth on body surfaces pose a serious health problem in the form of infectious diseases and substantial complications in clinics, sometimes even leading to death [1]. In recent decades, considerable attention has been paid to developing antibacterial fabrics to reduce the extent of initial bacterial attachment and thereby prevent bacterial growth and subsequent complications [2]. This technology is also gaining importance in biomedical technology as an alternative solution to increasing bacterial resistance. Conventional fabrics like cotton and nylon are widely converted to smart fabric by multiple approaches and the converted smart textiles possess the properties of conventional textile materials and carry additive functional values [3]. Our group has already reported the chitosan- cross-linked cotton fabric as a durable antibacterial fabric [4] and there we achieved anti-bacterial activity till 30 washing cycles. The present study is a step forward in the direction as we have incorporated silver nanoparticles into the chitosan modified cotton and cotton-nylon fabrics to enhance the antibacterial potential of the fabrics up to 50 washing cycles. Many research projects are dedicated to exploring and developing smart textiles for medicine and healthcare applications [5]. The use of smart textile materials is as an asset in everyday activities and accomplish better outcomes in healthcare systems. Generally, antibacterial fabrics are used in baby garments, clothes of individuals at higher risk of getting infections (staff of healthcare system and individuals with compromised immune system), and surgical clothes [6].

To develop an antibacterial fabric, several antibacterial agents have been used, such as chitosan [7], metal oxides like ZnO [8] and graphene oxide [9], and metal-based nanoparticles [10]. Among all these antibacterial agents, nanoparticles are most widely used because of their large surface area and remarkable antimicrobial properties [11]. In this study, we used silver nanoparticles (AgNPs) that have been extensively used in commercially available fabrics, such as clean room

materials, surgical gowns, and bandages. Silver has been used as a disinfectant and antimicrobial substance since it has a low risk of side effects [12]. Silver nanoparticles can penetrate bacterial cell walls, altering the shape of cell membranes and even causing cell death. They have a wide range of antibacterial, antifungal, and antiviral activities [13]. Despite all the advantages of nanoparticles their firm attachment on the surface of the fabric is a challenge and poor or weak attachment leads to decreased antibacterial efficacy, particularly after washing. Chan et al., reported that after 20 washing cycles, the coating of NPs was mostly diminished, because of their poor attachment or bonding with the fabrics [14]. To address this challenge numerous methods like pad-dry-cure, exhaustion, plasma direct synthesis, the use of polymeric binders and ultrasound have been exploited to achieve good attachment [15]. The polymeric binders can accomplish two tasks simultaneously first linking with fabric and secondly immobilizing the NPs and some binders, such as polydopamine, amino-terminated hyperbranched polymers (HPB-NH₂) and carboxymethyl chitosan (CMC) have been studied well [16]. In addition to this, urea, polyacrylic esters, polyurethane resins and dimethyl dihydroxy-ethylene urea have been applied to increase the affinity of NPs on the fabrics to enhance the antibacterial durability. But these binders are toxic to the human body and the environment as well [2]. Chitosan is well-known biomaterial and widely used in biomedical applications owing to its biocompatibility, biodegradability, and anti-microbial effects [17]. For the attachment of chitosan with fabric triethyl orthoformate (TEOF) was used and TEOF is reported by our group as a crosslinking agent [18].

In current research, we used chitosan as a binder to keep the AgNPs on the fabric surface with coordination covalent bonds resulting in the desirable prolonged antibacterial performance. Nanoparticle incorporation on the fabric was confirmed through Scanning electron microscopy (SEM), Fourier transforms infrared (FTIR) spectroscopy, and X-ray diffraction analysis (XRD). The nature of modified fabrics including mechanical properties, such as tensile strengths was measured and compared with the original fabrics. The antibacterial activity and laundering durability of the AgNP-incorporated fabrics were evaluated by using disc diffusion and broth dilution methods over 50 cycles of washing. Based on the findings, we are convinced that the developed approach is practically sustainable to produce a durable, antibacterial smart fabric that has multiple uses.

2. Material and Methods

2.1. Materials

Chitosan (Molecular weight: 25 KDA, Degree of deacetylation: 87%, and Intrinsic Viscosity 30.78ml/g) was prepared in our laboratory. Cotton fabric 100% (0.2 mm thickness, 1.5m length, and 1m width) was bought from the Burewala Textile Co., Ltd Pakistan. Cotton/nylon (50:50) fabric (35-inch width, 58-inch length,) was purchased from the Al-Rahim Textile Co., Ltd Pakistan. Nylon (85%) fabric (30-inch length, 24-inch width) was purchased from the local market of Lahore, Pakistan. Sodium hydroxide in pellet form was purchased from Omicron Sciences Limited. Glacial acetic acid (99% pure) and silver nitrate were purchased from Sigma Aldrich. Sodium borohydride (25g) was purchased from Daejung Chemicals and Metals Co., Ltd. Triethyl orthoformate (TEOF) (98%) was purchased from Alfa Aesar. Nutrient broth and nutrient agar were purchased from Sigma Aldrich.

2.2. Synthesis of silver nanoparticles

Silver nanoparticles were prepared according to the procedure reported in the literature [19]. Firstly, 1mM of AgNO_3 solution was prepared by dissolving 0.0169g of silver nitrate in a beaker containing 100mL of deionized water in an ice bath with continuous stirring for 30 minutes. Secondly, a 2mM aqueous solution of NaBH_4 was prepared separately and kept in an ice bath for 30 minutes. To synthesize AgNPs, chilled NaBH_4 was added dropwise to the colourless cold solution of AgNO_3 (1 mM) while maintaining stirring, until the light-yellow colour was achieved.

2.3. Chitosan coating for fabric modification

First, the alkali treatment was given to the nylon/cotton fabric. The $4 \times 4 \text{ cm}^2$ pieces of the fabric were immersed in the round bottom flask containing 1 g of NaOH in 100 ml water refluxed for 90 minutes [20]. After the cleaning process, the fabrics were dried in an oven at 80°C and then dipped into the 1 ml of 2% chitosan solution (which was prepared in 1% CH_3COOH (v/v) solution) along with 1 ml TEOF crosslinker (2.5%) for overnight, the time duration of dipping for cotton/nylon blended and nylon fabrics was 48 hours. After this, the fabrics were taken out from the solution and dipped into 1% acetic acid solution for washing and then dried at room temperature (37°C) [21].

2.4. Immobilization of AgNPs on the fabric surface crosslinked with chitosan

AgNPs produced by NaBH_4 reduction of Ag^+ ions were impregnated into the chitosan crosslinked fabrics by physical loading method. The modified fabrics were dipped into the light-yellow

solution of silver nanoparticles for 24 hours at 0 °C [22]. After that, the fabrics were removed from the solution and dried at room temperature (37 °C). After synthesis, the 6 samples (codes and description in Table 1) were used for characterization and testing.

Table 1. Codes and description of all prepared fabrics.

No.	Codes	Description
1	Cot	Plain cotton fabric
2	Nyl	Plain nylon fabric
3	Cot-Nyl	Plain cotton and nylon fabric
4	Ag-Cs-Cot	Silver nanoparticles incorporated chitosan modified cotton fabric
5	Ag-Cs-Nyl	Silver nanoparticles incorporated chitosan modified nylon fabric
6	Ag-Cs-Cot-Nyl	Silver nanoparticles incorporated chitosan modified cotton and nylon fabric

2.5. Characterization of smart fabrics

2.5.1. Fourier Transform Infra-red spectroscopy (FTIR) analysis

FTIR analysis was performed to investigate the chemical structures by using Thermo Nicolet 6700™ FTIR, equipped with an Attenuated Total Reflectance (ATR) sampling accessory having the germanium crystal. Spectra were obtained within the spectral range of 4000 – 650 cm⁻¹ at 8 cm⁻¹ resolution by accumulating the 256 scans.

2.5.2. Morphological analysis by Scanning Electron Microscope (SEM)

For morphological analysis of the developed smart fabrics was done through SEM. The microscopy was performed at a voltage of 10 KV at room temperature. The images taken by microscopy were then processed through Image J software.

2.5.3. Structural analysis by X-ray diffraction analysis (XRD)

Developed smart fabrics were characterized by the X-ray powder diffraction using Rigaku mini flex 600c diffractometer. For XRD analysis 40KV and 40 mA operational parameters were using radiation and with a step size of 0.02° range of 2 theta angle was from 20° to 90°.

2.5.4. Mechanical behaviour analysis by tensile test

Uniaxial tensile testing of the untreated and treated fabric samples was carried out using an electrodynamic fatigue testing system (1.5kN Load cell, Walter+bai, LFV-E, Switzerland) according to the guidelines in ISO 13934-1. The fabric specimens were cut into dumbbell-shaped geometry keeping the length and width same for each sample (20 mm gauge length and 10 mm width) and placed into the tensiometer grips. The materials were strained at a crosshead speed of 5mm/s and ultimate tensile strength was measured at the failure point. Mechanical strength data was obtained by plotting the stress against percentage strain. In each case, samples were analyzed in triplicates, and average values were obtained.

2.6. Anti-bacterial activity of smart fabrics

2.6.1. Testing of the antibacterial ability of smart fabrics

The antibacterial activity of the silver nanoparticles loaded fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) was evaluated qualitatively and quantitatively by disc diffusion and broth dilution method respectively while the plain fabrics (Cot, Nyl, and Cot-Nyl) were used as control. In both analyses, *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) were taken as model bacteria.

2.6.1.1. Anti-bacterial and durability studies of fabrics after washing

The antibacterial activity of fabrics was assessed by agar plate method [23]. The bacterial strains were grown in a nutrient broth medium overnight time. The cultures from broth (containing $\sim 10^6$ CFU/mL) were then swabbed on nutrient agar plates by sterile cotton swabs. Sample fabrics were cut into discs of 5mm diameter size and placed on the nutrient agar plates in triplicates. The plates were incubated at 37 °C for 24 h and examined. The appearance of a clear zone around the sample was recorded as a sign of bacterial inhibition against tested bacterial strains. The extent of the diameter of the inhibition zone was taken as a relative measure. The means of triplicates were calculated.

To check the antibacterial durability after repeated washing cycles, the samples were washed with non-ionic detergent (10 g/L). The samples were stirred for 15 minutes at the speed of 100rpm at room temperature. After that, the samples were rinsed with tap water and then air-dried. This procedure was repeated 50 times. The antibacterial activity of these samples was checked after 10, 20, 30, 40, and 50 washing cycles.

2.6.1.2. Anti-bacterial and durability studies after washing by broth dilution assay

To quantitatively investigate the anti-bacterial ability of fabrics broth dilution method was used[24]. To investigate the anti-bacterial ability and durability after washing the modified fabrics, $\sim 10^6$ CFU/mL of tested bacterial strains were grown in 15 ml nutrient broth. The sample fabrics were cut into 2mm discs and placed in the media and placed in the incubator for 48 h at 37 °C. As the negative control, no sample was added to the bacterial suspensions. The killing rate of bacteria and the bacterial concentrations were analysed by measuring the optical density (OD 600 nm) at regular intervals. All samples were evaluated in triplicates. The following equation was used to measure the % bacterial growth.

$$\% B.G = A/B \times 100$$

Where *B.G* is the rate of bacterial growth, *A* is the number of bacterial colonies from treated and untreated fabric and *B* is the number of bacterial colonies from the negative control.

To check the antibacterial durability after repeated washing cycles, the samples were washed with non-ionic detergent (10 g/L). The samples were stirred for 15 minutes at the speed of 100RPM at room temperature. After that, the samples were rinsed with tap water and then air-dried. The whole procedure was performed 50 times and the antibacterial activity of the samples was checked after every 10 cycles of washing (10, 20, 30, 40, and 50).

2.6.2 Analysis of the antibacterial ability of smart fabric after the football game

The patches of plain fabrics (Cot, Cot-Nyl) and AgNPs incorporated fabrics (Ag-Cs-Cot and Ag-Cs-Cot-Nyl) were stitched to the socks of players of football. The duration of the play was 70 minutes. After the game, the patches were removed from the socks bacteria were counted on the plain fabrics, and AgNPs incorporated fabrics by the broth dilution method. The fabric samples (which were attached to player's socks) were dipped into the broth medium and then incubated at 37 °C for the time of 24 hours. The growth of bacteria was assessed by measuring optical density (OD 600 nm).

3. Results

3.1. Preparation of silver nanoparticles

For preparation different reaction conditions (reagents, time of reaction, and temperature) were tried (Table 2) and silver nanoparticles were successfully prepared by the reducing action of sodium borohydride with stabilizer PVA/PVP 0.3%, reaction time of 5 minutes at 0°C temperature.

The color changes of the solution from colorless to light yellow and finally yellowish-brown confirmed the formation of silver NPs (surface plasmon excitation vibrations in metal nanoparticles caused the intense yellowish-brown color) with reference from literature data [25, 26]. Further confirmation of the formation of AgNPs was characterized by UV-vis spectroscopy. The solution absorbed the light in the range of 300-600 nm [27] and strong broad peaks were observed at $\lambda_{\max} = 424$ nm in (figure 1A

Table 2: Different methods for the formation of silver nanoparticles.

Sr. No.	Reagents			Time of reaction	Temperature	Results
	Substrate	Reducing agent	Stabilizer			
1	AgNO ₃ 0.1 M	Ascorbic acid 4.635×10^{-4} M	Citric acid 2.781×10^{-3} M	30 Minutes	37°C	Silver nanoparticles were not formed
2	AgNO ₃ 0.01M	NaBH ₄ 0.02M	PVA/PVP 0.3%	15 Minutes	37°C	Silver nanoparticles was not formed
3	AgNO ₃ 1×10^{-3} M	NaBH ₄ 2×10^{-3} M	—	5 Minutes	0°C	Silver nanoparticles were formed

3.2. Assessment of morphological features of prepared silver nanoparticles

Transmission electron microscopy was used to investigate the morphological features of as-prepared spherical shape silver nanoparticles (Figure 1B). The particle size of as-prepared nanoparticles was found in the overall range of 10-20 nm. This inhomogeneous size of prepared silver nanoparticles could be attributed to more easy heterogeneous nucleation in liquid phase. Because of their surface morphology and small size of the silver nanoparticles, they are expected to display outstanding activities[28].

3.3. Preparation of smart fabrics

The commercially available cotton, nylon, and cotton-nylon fabrics were modified by coating of chitosan with the crosslinking of TEOF, and the prepared NPs were incorporated into the chitosan modified cotton/nylon fabrics (figure 1B). Most fabrics have negative charge on them due to hydroxyl groups that help them to attach with positive charge of silver in silver nanoparticles [29]. Moreover, the different functional groups of fabric, chitosan and silver nanoparticles play their role in strong bonding and sustainable loading of silver nanoparticles on fabrics [30]. The reactions of hydroxyl groups of the cotton, amine groups of the nylon and cotton/nylon fabrics, and the amines group of the chitosan with TEOF resulted in imine functionality, which created a covalent bond between the fabrics and chitosan. Then the amine- and hydroxyl- groups of chitosan made the coordination covalent bonds with AgNPs (figure 2).

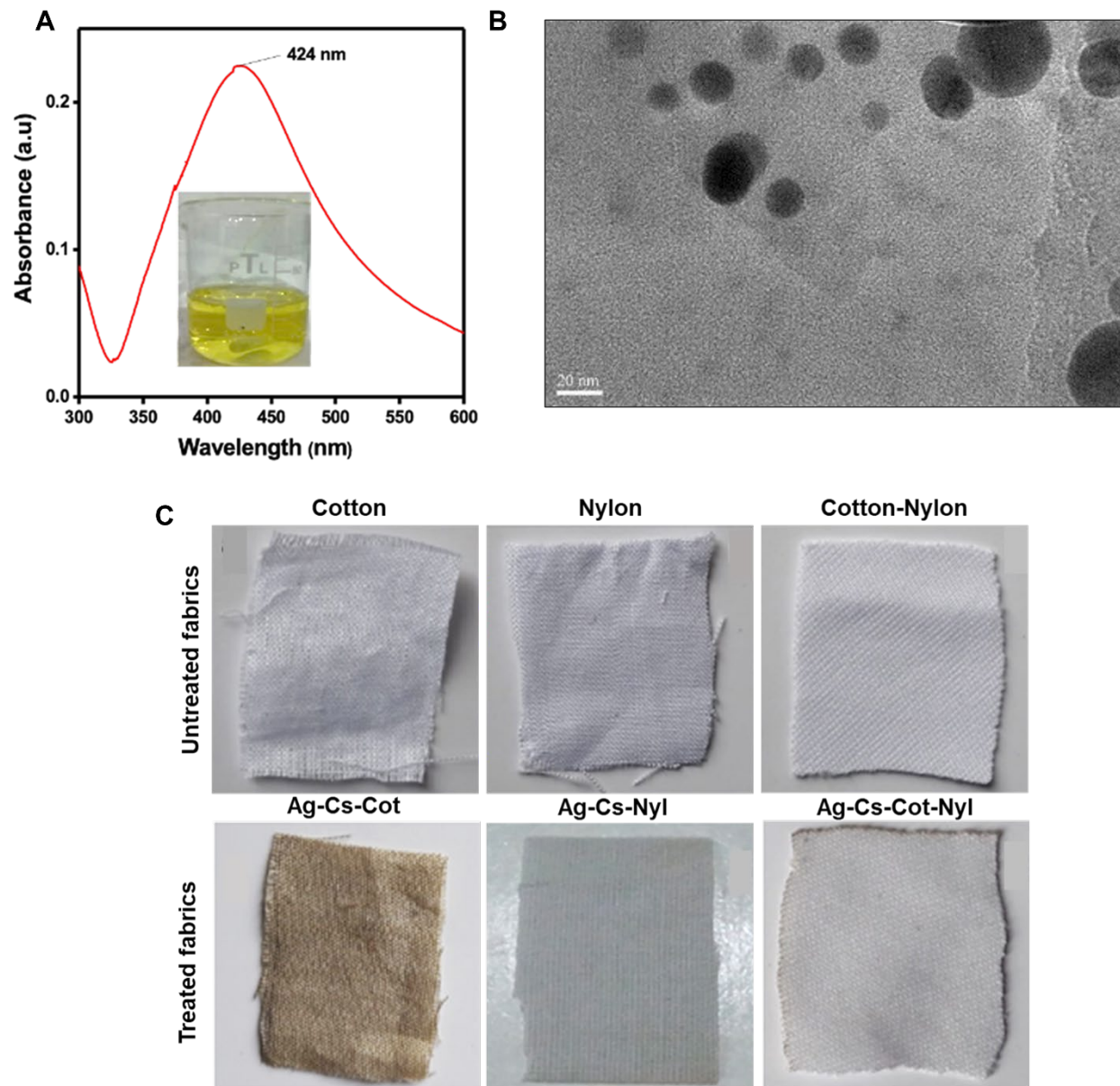


Figure 1. UV-vis spectroscopic confirmation of synthesized AgNPs. B. TEM images of silver nanoparticles. C. Schematic presentation of the modification of conventional fabric and loading of AgNPs on modified fabrics. C. Original photographs of plain and NPs loaded fabrics.

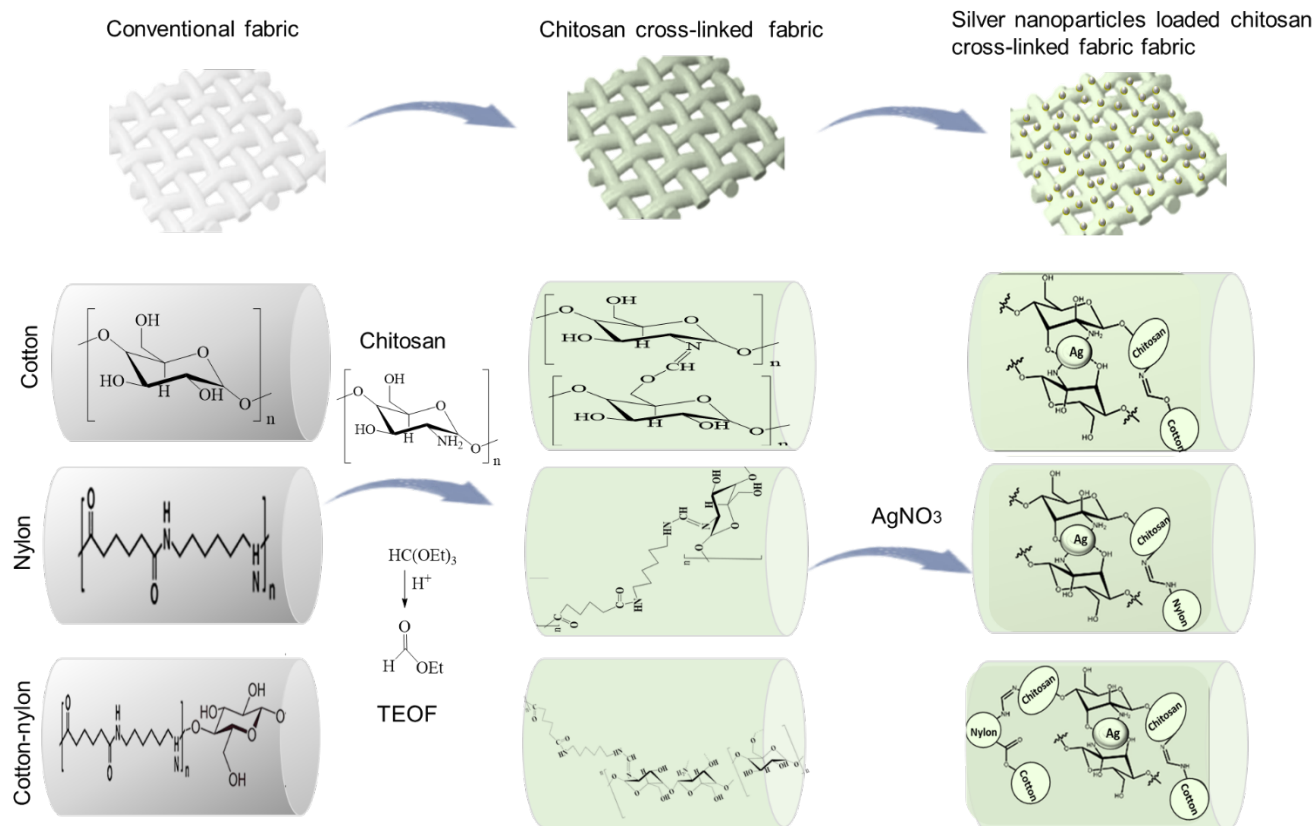


Figure 2: The chemical reaction scheme of chitosan cross-linked silver nanoparticles loaded cotton, nylon, and cotton-nylon fabrics.

3.4. Chemical analysis by Fourier Transform Infra-red spectroscopy (FTIR)

FTIR analysis was performed to confirm the chemical interactions among chitosan, TEOF, and conventional fabrics. For each type of fabric (cotton, nylon, and cotton-nylon) FTIR analysis was run separately and the results are presented here below.

3.4.1. Confirmation of chitosan crosslinking with fabric by TEOF

Firstly, we tested the crosslinking of chitosan with the fabric with and without TEOF. The resulting fabrics were washed with the acetic acid, as chitosan is soluble in acetic acid, if chitosan was detached during washing it means it was not attached chemically with the fabric to confirm that FTIR analyses were carried out. Results (figure 3A) showed that chitosan with TEOF peak at 1650 cm^{-1} is an indicator of the $\text{C}=\text{N}$ which was the Schiff base. It means chitosan was covalently attached to the fabric due to the crosslinking ability of TEOF. On the other hand, the fabric without TEOF showed no peak at 1650 cm^{-1} which means the chitosan was washed away with the acetic acid.

3.4..1. FTIR results of cotton fabric

The FTIR results of cotton fabric are shown in Figure 3A in which a comparison of the results between the plain and the silver nanoparticles loaded cotton fabric is presented with the confirmation of nanoparticles loading on the fabric. The absorption peak at 3336 cm^{-1} corresponds to the O-H functional group of the cotton and chitosan. The peaks observed at 1149 cm^{-1} and 897 cm^{-1} were present in the C-O stretching while the peak observed at 1578 cm^{-1} is of NH_2 . The peak at 1161 cm^{-1} presents the ether linkage C while the peak at 2850 cm^{-1} showed the CH_2 stretching. The peak at 1650 cm^{-1} presented the C=N Schiff base formation peak that indicated the covalent attachment of chitosan with cotton fabric. A peak near the 3375 cm^{-1} indicated the secondary amine bending that showed the silver nanoparticles' attachment with the chitosan through the bonding with the amine group of chitosan.

3.4.2. FTIR results of nylon fabric

Confirmation of AgNPs loading on the nylon fabric through the crosslinking of chitosan and TEOF is shown in Figure 3B. The absorption peak at 2900 cm^{-1} presents the O-H functional group present in nylon and chitosan. The peak observed at the 710 cm^{-1} was of CH_2 rocking. The 1578 cm^{-1} peak was of NH_2 functional group present in chitosan. The peak observed at 1350 cm^{-1} was of C-O while the peak observed at 1710 cm^{-1} was of C=O. The ether linkage C-O-C showed a peak at 1161 cm^{-1} while the CH_2 stretching vibration showed a peak at 1429 cm^{-1} . The peak at 1650 cm^{-1} showed the C=N Schiff base as the confirmation of chitosan covalent attachment with the nylon fabric. The peak near the 3315 cm^{-1} indicated the attachment of silver nanoparticles to the chitosan.

3.4.3. FTIR results of nylon-cotton fabric

Confirmation of AgNPs attachment to the nylon-cotton fabric through the crosslinking of chitosan and TEOF is shown in figure 3C. The absorption peak of 2900 cm^{-1} presents the O-H functional group of nylon-cotton and chitosan. The peak observed at the 970 cm^{-1} was of CO-NH while the peak observed at 1578 cm^{-1} was of NH_2 group present in the chitosan. The C-N peak appeared at 1350 cm^{-1} while C=O peak was appeared at 1710 cm^{-1} . At 2850 cm^{-1} CH_2 stretching was observed that confirmed the Schiff base formation and indicated the chitosan covalent attachment with the cotton-nylon fabric. The peak near the 3375 cm^{-1} indicated the attachment of the silver nanoparticles with the amine functional group of cross-linked chitosan.

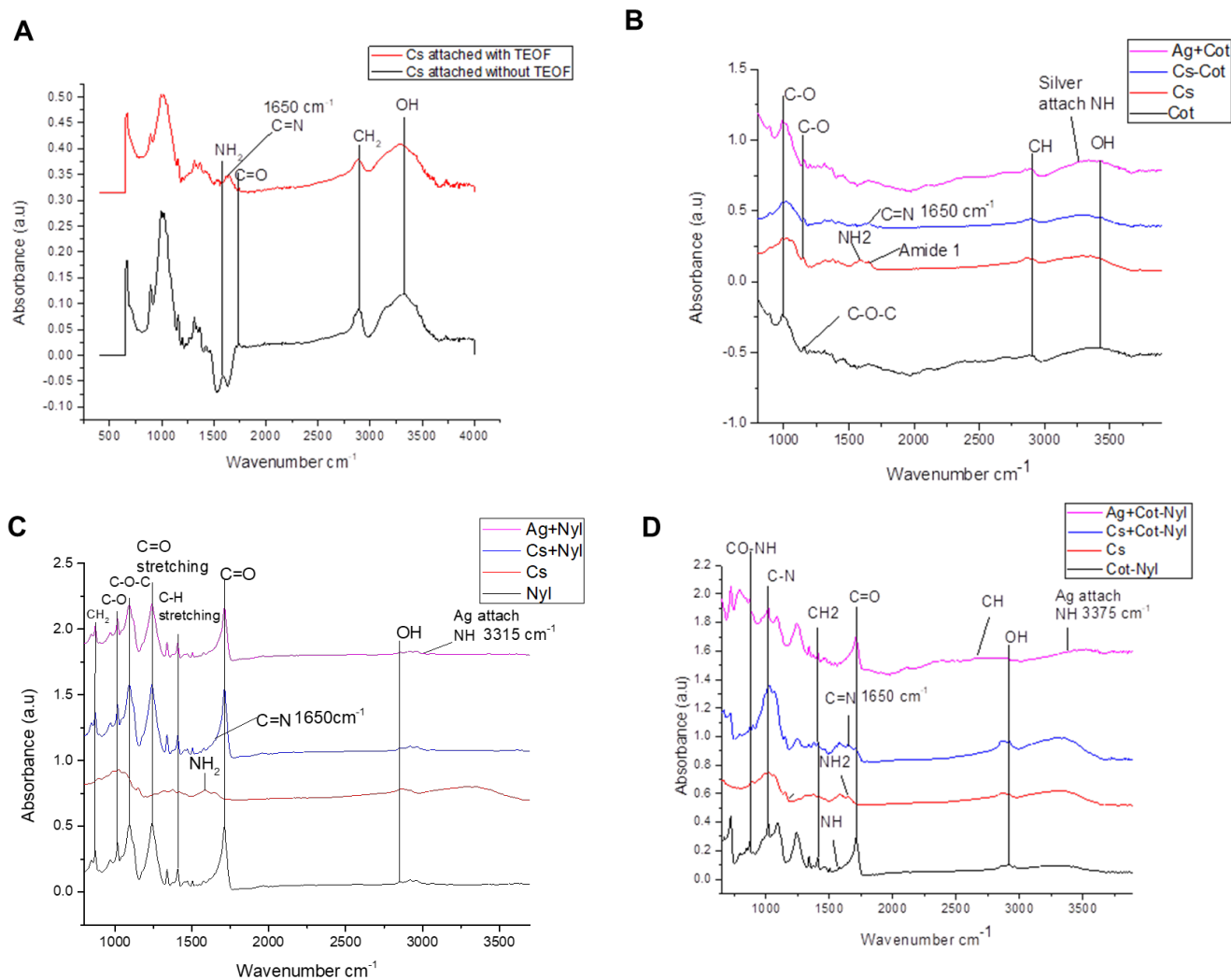


Figure 3. FTIR spectra of developed smart fabrics. A. Confirmation FTIR spectra of TEOF role in chitosan crosslinking with fabric. B. FTIR spectra of cotton fabric and its modifications. C. FTIR spectra of nylon fabric and its modifications. D. FTIR spectra of cotton-nylon fabric and its modifications.

3.5. Structural analysis by X-ray diffraction analysis (XRD)

XRD analysis of developed fabrics was performed to investigate the structure of the smart fabrics. Diffraction planes at 20° represent the presence of C in the fabrics. Peaks of silver nanoparticles are usually observed at 38.2901° and 44.5583° but in our study well resolved sharp peaks of silver nanoparticles have been observed at $21.32^\circ, 23.15^\circ$ due to their binding with fabrics. Peaks at $20.56^\circ, 20.7^\circ, 21.32^\circ, 23.15^\circ$ represents the amorphous surface of fabric due to carbon presence. Phase purity and crystallinity of synthesized Ag-Cs-Cot (A), Ag-Cs-Nyl (B) and Ag-Cs-Cot+Nyl (C) were

also investigated. These finding implies that there is no significant change in the XRD results of fabrics before and after the loading of silver nanoparticles. The principal diffraction peaks for Ag with diffraction plane (111), (200), and (220) at diffraction angle of 38.2, 44.5 and 67.4°, respectively matched well with the JCPDS no: 04-0783. Whereas diffraction peaks around the 20-30° represents the presence of C in the fabrics. The diffraction peaks (110), (200), (020), and (002) at diffraction angle of 20.56, 20.7, 21.32, and 23.15° represents the amorphous surface of fabric due to carbon presence (Figure 4A).

3.6. Mechanical behavior analysis by tensile test

The mechanical behavior of all 6 fabrics was tested in the (Figure 4B). The modification with chitosan and incorporation of AgNPs altered the strength and mechanical characteristics of the fabrics. In cotton fabric tensile strength of the fiber increased as plain cotton fabric showed tensile strength of 13 MPa while the chitosan modified silver nanoparticles loaded cotton fabric (Ag-Cs-Cot) showed tensile strength of 18 MPa. Similarly, an increase in the tensile strength in both nylon and cotton/nylon was observed. In nylon, the tensile strength was increased to 100 MPa from 90 MPa, and in cotton-nylon, the tensile strength was increased to 99 MPa from 91 MPa after the modification with chitosan and incorporation of AgNPs. Chitosan greatly enhanced the mechanical strength of fabric as it is known for its great mechanical strength [31] and it is used to enhance the mechanical strength of the paper [32]. Moreover, silver nanoparticles increased the mechanical strength of fabric as it is already cited in literature that silver increases the mechanical strength of polymers [33, 34] due to its good mechanical strength (125 MPa for 5 mm wire) [35].

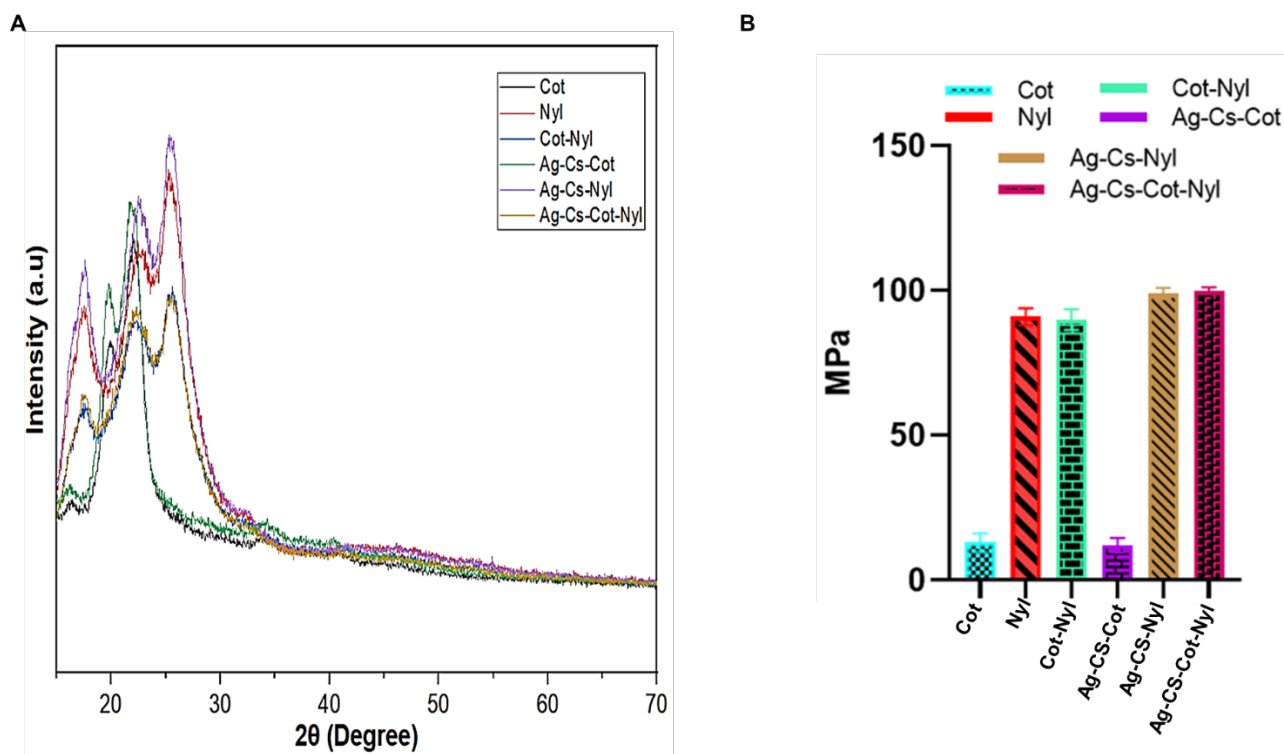


Figure 4. A. XRD spectra of synthesized smart fabrics. B. Tensile strength measurements of smart fabrics.

3.7. Morphological analysis by Scanning Electron Microscope (SEM)

The prepared fabrics were characterized by the SEM for their morphological analysis. The images of plain fabrics (Cot, Nyl, and Cot-Nyl) and the silver nanoparticles loaded fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) showed smooth surfaces (figure 5). In smart fabrics images the smooth surface appearance of fabric indicate the well-dispersed coating of nanoparticles in all three fabrics ((Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl). Similarly, a previous study has reported smooth SEM images of AgNP loaded cotton fabric [36]. The smooth and even surface of fibers indicates the fact that chitosan crosslinking or silver particle loading did not degrade the fiber structure.

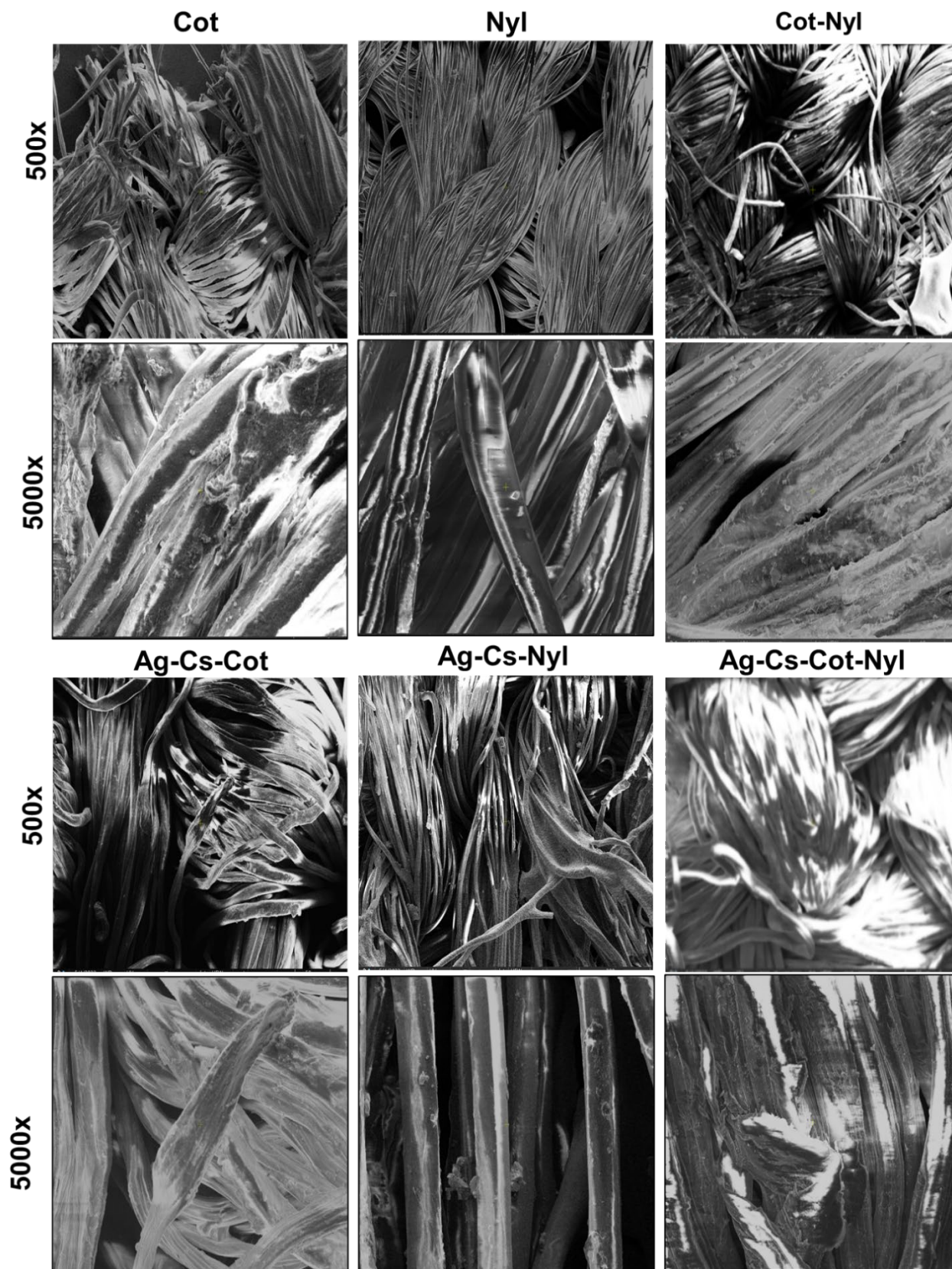


Figure 5. SEM images of plain and NPs loaded fabrics at 500X and 5000X.

3.8. Testing of the antibacterial ability of smart fabrics

Antibacterial ability of developed smart fabric was tested by the agar diffusion method. This strong antibacterial potential of the AgNPs loaded smart fabric was observed in this experiment as a good inhibition zone in all three AgNPs loaded fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) against the plain fabrics (Cot, Nyl, and Cot-Nyl) is shown in figure 6A.

3.9. Anti-bacterial and durability studies after washing by agar diffusion assay

Agar diffusion method was used to qualitatively analyse the antibacterial ability of AgNPs loaded smart fabrics. The growth of the bacteria was checked by the zone of inhibition method. Plain fabrics (Cot, Nyl, and Cot-Nyl) showed no zone of inhibition indicating no antibacterial activity. The AgNPs loaded fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) showed the inhibition zones as an indication of the antibacterial ability of the fabrics. The antibacterial ability of the fabrics was assessed after the washing to check the durability of the antibacterial ability. Results showed that the anti-bacterial activity of smart fabrics was retained after 50 washing cycles against both gram-positive and gram-negative bacteria, though the zone of inhibition was decreased with every washing (figure 6 B).

3.10. Quantitative analysis of anti-bacterial ability and durability after washing by broth dilution assay

Quantitative analysis of anti-bacterial ability and durability of developed smart fabrics was checked by investigating the growth of bacteria in nutrient broth by taking OD measurements at regular intervals for 48 hrs. After 48 hrs all AgNPs loaded fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) showed significant killing ability against both bacterial strains, and the highest antibacterial reduction was observed in the Ag-Cs-Cot-Nyl that is 93 % bacterial reduction against *E. coli* and 89.7 % reduction against *S. aureus* (figure 6 C). Ag-Cs-Cot showed 90% bacterial reduction against *E. coli* and 78.7 % reduction against *S. aureus* while the Ag-Cs-Nyl showed 88% bacterial reduction against *E. coli* and 75 % reduction against *S. aureus*. The antibacterial durability of the smart fabrics was also checked over the 50 washing cycles. The smart fabrics retained the antibacterial ability till the 50th washing though a marginal decrease was observed in their antibacterial ability. A 78 % reduction for *S. aureus* and 82 % for *E. coli* after 48 hrs was observed after 50 washing cycles (figure 6 D).

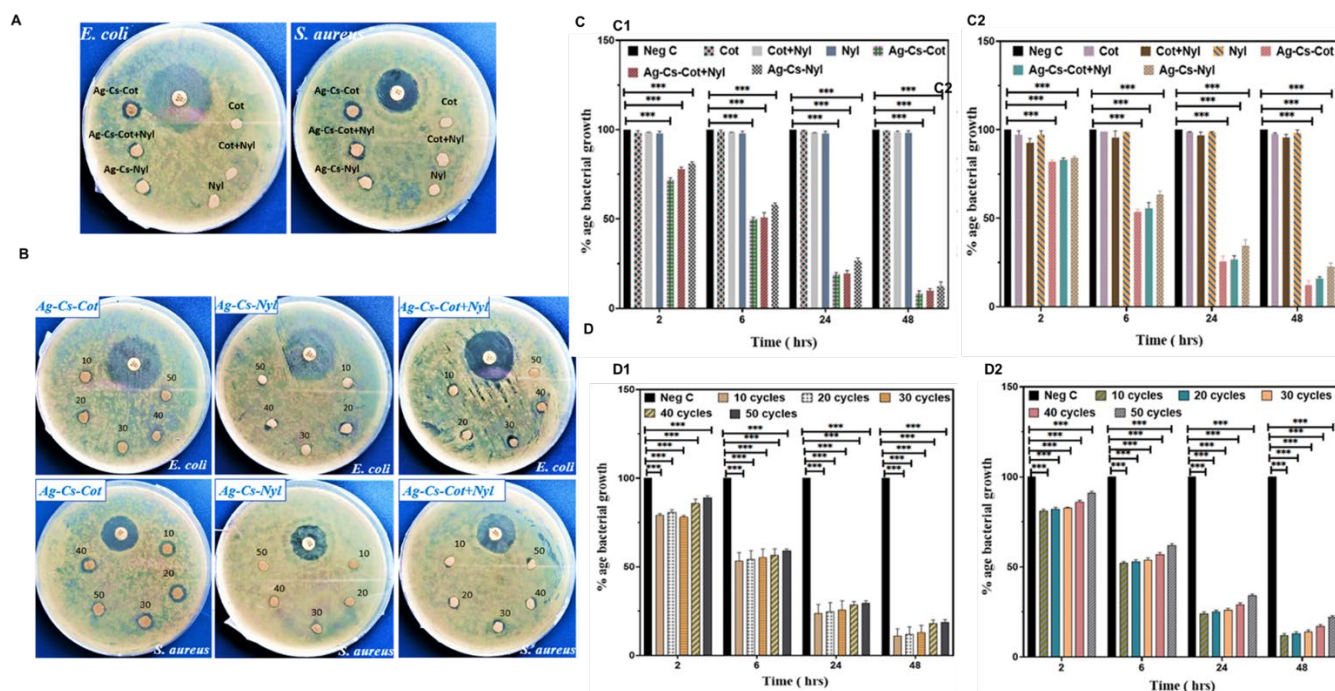
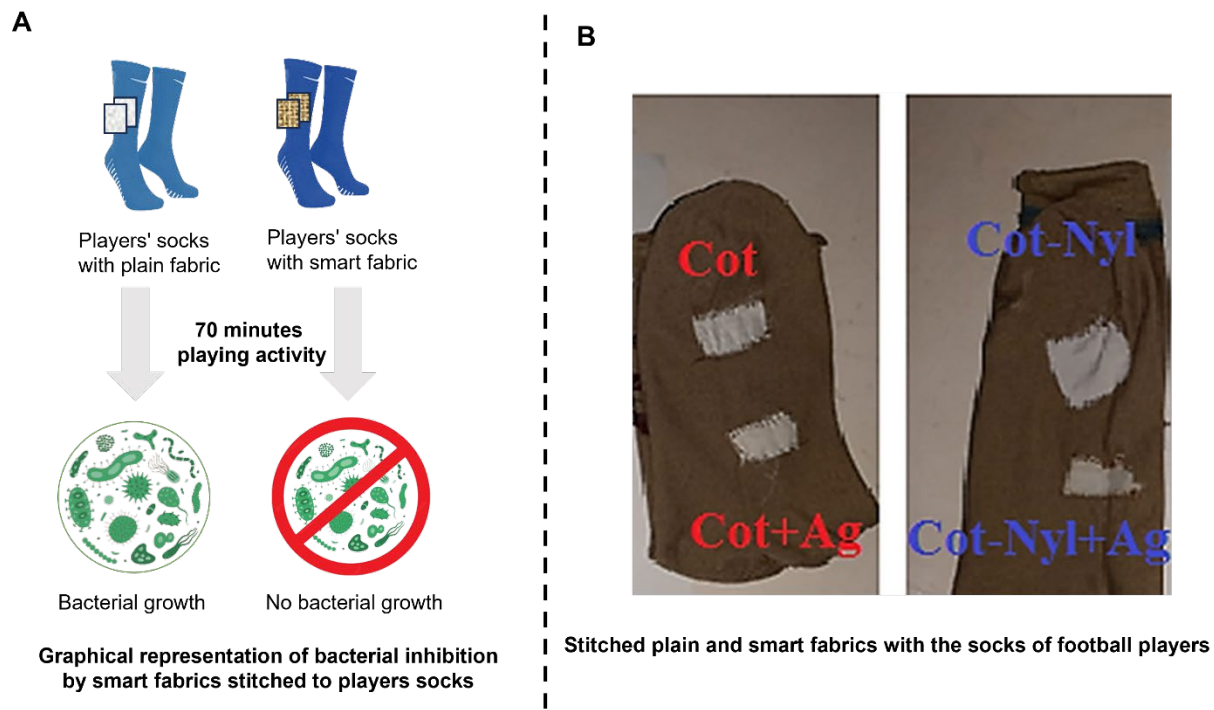


Figure 6. **A.** Antibacterial ability of developed smart fabrics before washing. **B.** Antibacterial durability of developed smart fabrics after the 50 washing cycles. **C.** Antibacterial ability of developed smart fabrics before washing. **C1.** Antibacterial ability of developed smart fabrics before washing against *E. coli*. **C2.** Antibacterial ability of developed smart fabrics before washing against *S. aureus*. **D.** Antibacterial ability of developed smart fabrics after washing. **D1.** Antibacterial ability of developed smart fabrics after washing against *E. coli*. **D2.** The antibacterial ability of developed smart fabrics after washing against *S. aureus*. One-way ANOVA analysis of variance implies a significant difference (***) ($P < 0.0001$).

3.11. Analysis of the antibacterial ability of synthesized smart fabric after the football game

Antibacterial ability of the synthesized smart fabric to control the growth of bacteria was further checked by attaching the synthesized smart fabrics to the socks of football players (figure 7B). After the football activity of 70 minutes, the fabrics were detached, and the antibacterial activity of fabrics was assessed by broth dilution method. Results (figure 7C) showed the good antibacterial ability of AgNP loaded fabrics as compared to the plain fabrics. The bacterial reduction was about 90 % by the AgNPs loaded fabrics. These results indicated that AgNP loaded fabrics possess good control over bacterial growth after 70 minutes exposure to bacteria.



C

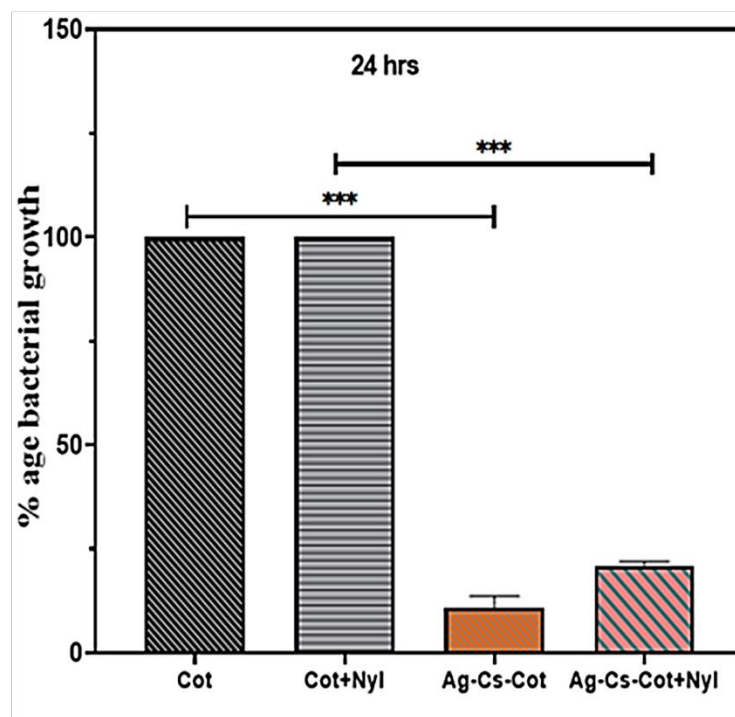


Figure 7. A. Graphical representation of bacterial inhibition by smart fabrics attached to players' socks. B. original photograph of stitched plain and smart fabrics with the socks of football players. C. Graphical representation of bacterial cells grown on plain and AgNPs loaded fabrics attached to the socks of the player. One way ANOVA analysis of variance implies significant difference (***) $P < 0.0001$).

4. Discussion

With the increasing risks of infections, the escalating level of antibacterial resistance, and augmenting burden on the healthcare system new and better anti-bacterial solutions are required. The demand of smart fabrics as the first line of defense against infections is increasing [37]. Due to the special qualities of textiles, including their light weight, flexibility, dimensional variability, and ability to be modified at different levels in terms of both structure and surface, multidisciplinary research in engineering and medicine is concentrating on key innovations in textiles [38]. There are numerous instances of successful attempts to increase the functionality, chemical and physical characteristics of textiles. Furthermore, a noteworthy advancement has been made in the past few decades in investigating and improving the potential of textiles to address various health concerns and hazards [39].

Developing an antibacterial smart fabric with sustained antibacterial ability is one such breakthrough in which numerous efforts have been invested [40, 41]. In different forms from normal to medical wear (for example clothes of babies and old persons, sportswear, surgical clothes, and professional garments of medical and healthcare workers) an antibacterial fabric can be a first line defence against the major bacterial infections of skin including Herpes Simplex [42, 43]. The antibacterial fabric can be used for multiple biomedical applications like face mask to inhibit respiratory infections, surgical gloves to protect the surgeons from getting/transmitting infections during critical operations, and scaffolds for wound healing dressing as the antibacterial ability of the fabric reduces the risk of bacterial infections which is a major challenge in the chronic wound management [44].

In last few years a number of studies have reported the modified fabrics having the strong antibacterial potential even after multiple washing cycles [45]. Researchers had reported more than 90% antibacterial potential of modified cotton fabric after 50 washing cycles [46]. Another report showed study had reported the good antibacterial potential of modified polyester fabric [47]. A similar study had reported the antibacterial activity of modified cotton fabric without any sustainable antibacterial ability [4, 48]. But in this study our aim was to present a range of fabrics for diverse range of applications. In this regard we modified three different fabrics cotton, nylon, and cotton/nylon blended fabric as these fabrics are most commonly used in normal and professional clothes. Overall, our synthetic procedure is easier to perform and we used the TEOF as crosslinker [49] to ensure the sustainable coating of chitosan on fabric that is distinguishing and novel feature of our study.

In this study we designed the nanotechnology-based approach to develop an antibacterial smart fabric with sustained antibacterial ability even after multiple washing (50 cycles) for biomedical applications. The conventional cotton/nylon fabric was used in this study as it is the most common textile for wear in normal and professional clothes [50]. The conventional cotton/nylon fabric was first modified by the coating of chitosan to make the surface convenient for nanoparticle attachment. Chitosan with the help of crosslinker TEOF made the covalent linkage with the amines of the cotton/nylon fabric and provided a good surface for strong attachment of nanoparticles with the fabrics [49]. Chitosan is a widely used biopolymer due to its nontoxic, nonallergic, biocompatible, renewable, biodegradable polysaccharide and best chelating property owing to its absorbing capacity of metal ions by the amino and hydroxyl groups in its structure [51]. Chitosan is also used in textile industry for wide range of purposes including anti-wrinkle, anti-static and anti-bacterial [52, 53], it is also used for surface modification of fabrics for desired physical, electrical or electrothermal characteristics [54, 55]. In this study we used chitosan not only to modify the surface of the fabric for nanoparticles attachment but also for the additional antibacterial feature because chitosan possesses antibacterial properties as reported in literature [56, 57]. The nanoparticles selected for this study were silver nanoparticles which are well-known for their antibacterial properties [58, 59]. Multiple studies have been conducted on the development of silver nanoparticles loaded fabrics (Table 3).

Table 3. Studies based on silver nanoparticles loaded smart fabrics.

No.	Nanoparticles	Antibacterial activity	Reference
1	Silver nanoparticles loaded on silk fabric	97.4% of activity against <i>E. coli</i> and 99.8% of activity against <i>S. aureus</i>	[60]
2	Silver nanoparticles loaded on cotton fabric	99.2% of activity against <i>E. coli</i> and 99.01% of activity against <i>S. aureus</i> , even after 20 wash cycles	[61]
3	Silver nanoparticles loaded on polyester fabric	80% antibacterial activity <i>E. coli</i> and 75 %	[62]

		of activity against <i>S. aureus</i>	
4	Silver nanoparticles loaded on thermosensitive cotton fabric	Fair antibacterial activity against <i>E. coli</i> and <i>S. aureus</i>	[45]
5	Silver nanoparticles loaded on the non-woven fabric	Fair antibacterial activity against <i>E. coli</i> , <i>S. aureus</i> and, <i>B. subtilis</i>	[63]
6	Silver nanoparticles loaded on cotton fabric	Fair antibacterial activity against <i>E. coli</i> after 30 minutes washing	[30]

The loading of silver nanoparticles on the fabric was the main aim of this study as the good loading and interaction of AgNPs with fabric was the core of antibacterial ability of the fabric and that was achieved through the chitosan crosslinking with the fabric through TEOF (figure 3 A). Without TEOF chitosan could not be covalently attached to the fabric and silver nanoparticles could not load on the fabric. TEOF is well-recognized crosslinker that is widely used to crosslink different polymers [18]. The loading of AgNPs on fabric was ensured through the characterization of the developed smart fabrics. FTIR results (figure 3 B, C, D) confirmed the chemical interaction of AgNPs with the chitosan modified fabric as an indication of their loading on the fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl). The peak at 1650 cm^{-1} corresponds to the C=N Schiff base formation peak which indicates that the chitosan covalently attaches to the cotton fabric and the secondary amine bending vibration absorbs near the 3375 cm^{-1} which indicates that the silver attached to the amine group of the chitosan. XRD results confirmed the structural changes in the smart fabrics due to the chemical interaction between the functional groups of the fabric and the silver nanoparticles. The morphological analysis further confirmed the homogeneous coating of the silver nanoparticles on the chitosan modified cotton/nylon fabrics. The loading of nanoparticles generally modifies the mechanical behavior of the fabrics [64]. In this study, silver nanoparticles loading on the fabrics enhanced the tensile strength of the smart fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl). Silver nanoparticles are reported for their ability to enhance the tensile strength of the polymer [65].

A recent study presented the enhancement of polyurethane's tensile strength by adding different concentrations of silver nanoparticle [33]. This too can be presented as the indication of the AgNPs loading on the fabrics. Moreover, the enhanced tensile strength of the fabric can be used as an additional benefit in terms of its durability [66].

The antibacterial ability of the developed smart fabrics was tested through agar diffusion assay and broth dilution assay against *E. coli* and *S. aureus* bacterial strains. Silver nanoparticles possess great antibacterial potential as they lead to the cell death of bacteria by causing cell damage (figure 8). Various studies have reported the antibacterial potential of silver nanoparticles and their loading on different scaffolds for antibacterial applications in numerous fields [13, 58, 65, 67]. The ability of AgNPs to pass through outer membranes allows them to accumulate in the inner membrane, where their adhesion to the cell causes destabilization and damage. This increases the permeability of the membrane, causes cellular content to leak out, and ultimately causes the cell to die. Additionally, it has been demonstrated that AgNPs can interact with proteins in bacteria's cell walls that contain sulphur, potentially rupturing the cell wall due to structural damage [67, 68].

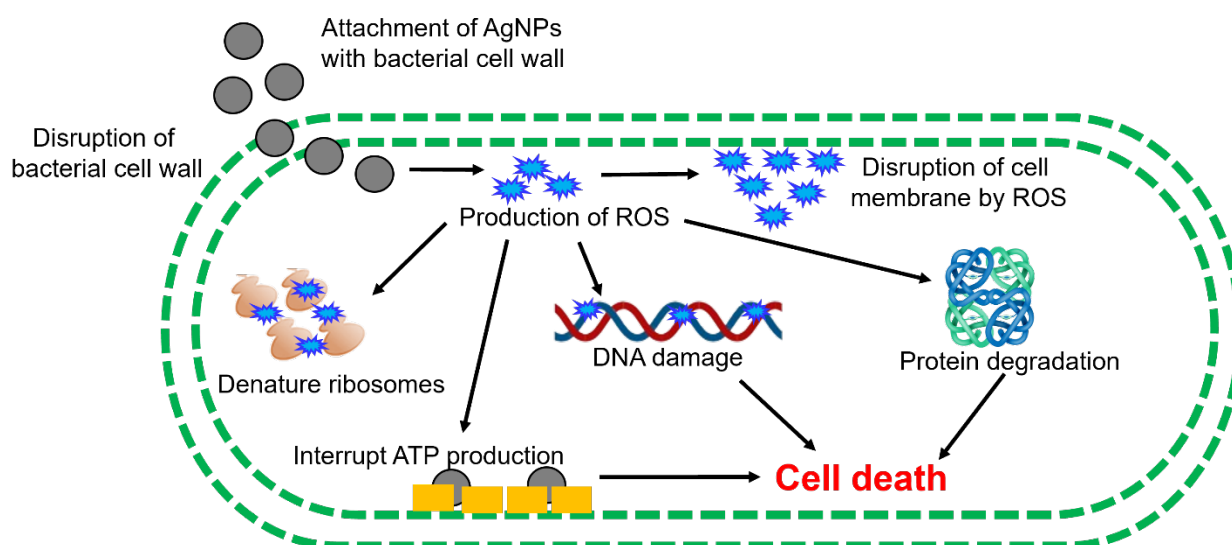


Figure 8. Antibacterial action of silver nanoparticles [69].

Results of both assays showed the good antibacterial ability of the developed smart fabrics. The smart fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) showed good bacterial inhibition against *E. coli* and *S. aureus*. Comparatively, the Ag-Cs-Cot-Nyl showed the highest antibacterial ability due to

better attachment of nanoparticles with the chitosan attached to the functional groups of nylon and cotton. The greater challenge in this regard was the durability of the antibacterial potential of the smart fabrics as the textiles are washed excessively and often lose the additional feature after washing. The durability of the antibacterial ability of the developed smart fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) was checked over the 50 washing cycles through the agar diffusion assay and broth dilution assay against *E. coli* and *S. aureus* bacterial strains. Results confirmed the retention of the antibacterial ability of the fabrics after the 50 washing cycles, though a 82 % reduction against the *E. coli* and 78 % reduction against the *S. aureus* was observed. However, retention of the antibacterial ability after the 50 washing cycles is a great deal. Results of our cotton/nylon fabrics are comparable to previously reported studies where the antibacterial potential of modified cotton and polyester fabrics was over 50 washing cycles [46, 47].

The antibacterial potential of the developed smart fabrics was also checked after the heavy exertion activity of football playing. The patch of fabrics, both plain and AgNPs loaded, were attached to the pair of socks of the player who played the football for 70 minutes. After 70 minutes the antibacterial ability of the fabrics was checked and results showed that the AgNPs loaded fabrics (Ag-Cs-Cot, and Ag-Cs-Cot-Nyl) showed 90% greater control over the bacteria as compared to the plain fabrics (Cot, Nyl, and Cot-Nyl) as hardly one or two colonies were observed in the petri plate of the AgNPs loaded fabrics while petri plates of the plain fabrics were full of the bacterial colonies. This indicated the strong bacterial control of the developed smart fabrics during the heavy sweating activities in the open, natural environment full of multiple bacterial strains causing severe life-threatening infections. Overall, the developed smart fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) depicted exceptional antibacterial potential against gram-negative bacteria and gram-positive in laboratory as well as in real-life experiments.

5. Conclusion

In summary, antibacterial fabrics with elongated antibacterial activity have been manufactured by the novel approach of loading silver nanoparticles on the chitosan modified cotton, nylon, and cotton-nylon fabrics. Crosslinker TEOF ensured chitosan coating on fabrics as depicted by FTIR results. Characterization of developed smart fabrics through SEM confirmed homogenous attachment and distribution of the AgNPs on the fabrics. Mechanical testing results confirmed good mechanical properties of the developed smart fabrics. Mainly, the qualitative and quantitative assessment of the

antibacterial activity of the developed smart fabrics (Ag-Cs-Cot, Ag-Cs-Nyl, and Ag-Cs-Cot-Nyl) was done and results showed significant bacterial inhibition against the *E. coli* (88-90%), and *S. aureus* (75-89.7 %). The most striking feature of the developed smart fabric is the retention of antibacterial potential over the 50 washing cycles. Moreover, the antibacterial ability of developed fabrics was assessed after 70 minutes playing activity as real-life testing of material and fabrics showed massive control (90 %) over bacterial growth. The findings of the study depicted the successful step towards the fabrication of smart fabrics with prolonged antibacterial activity. Based on these findings, the fabricated smart fabrics have great potential to be used in multiple applications.

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