Silver Decorated Bacterial Cellulose Nanocomposites as Antimicrobial Food Packaging Materials

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Abstract

This study is aimed to develop bacterial cellulose (BC)-based biocompatible, biodegradable, bioactive, and non-toxic food packaging material. The preparation of BC/Ag nanocomposite was achieved through the reduction of silver nitrate with sodium chloride. Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) analyses confirmed the purity of BC and the development of BC/Ag nanocomposite. SEM analysis showed the uniform distribution of Ag nanoparticles in the BC matrix, which further improved the water solubility to 4.6% and tensile strength to 25.7 MPa of BC/Ag nanocomposite. The developed BC/Ag nanocomposite did not show any toxicity towards NIH-3T3 fibroblasts. The BC/Ag nanocomposite showed antimicrobial activity against three bacterial strains (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Trichosporon sp.*) by producing inhibition zones of 0.17 cm, 0.08 cm, 0.16 cm, 0.06 cm, and 0.08 cm, respectively after 24 h. The BC/Ag nanocomposite film-coated oranges and tomatoes demonstrated acceptable sensory features such as odor and color at different storage temperatures for up to 9 weeks. These findings demonstrate that the BC/Ag nanocomposite film could be used as biocompatible packing material for providing protection and extending the shelf-life of different foods.

Keywords: Bacterial cellulose; Ag nanoparticles; Antimicrobial activity; Biocompatibility; Food packaging.

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1. Introduction

In recent years, consumers have increased the intake of minimally processed food and ready-to-eat items with a fresh and balanced attribute. Both food quality and safe packaging are the main requirements for fresh and ready-to-eat food items in the food industry. The continuous microbial outbreaks and increased resistance to conventional antimicrobials have continued to look for new ways of preventing microbial

growth on food while preserving its nutritional quality, freshness, and safety.^[1] The food processing technologies are mainly focusing on preventing the development of surface waste, moisture loss, browning, and proliferation of spoilage-related microorganisms by developing approaches like skinning, washing, dicing, and scrubbing operations.^[2] The microbial growth on surfaces of food items is mainly associated with their pH, water activity, composition, oxygen concentration, and redox capacity.^[3] Therefore, packaging plays an important role in food safety and preservation. Over the last couple of decades, the potential of various edible films and coatings has been extensively explored for improving the quality and safety of food items, with the aim to replace the conventional packaging products and processes. The materials used for packaging or coating of food items could be edible or non-edible depending upon their intrinsic properties, such as toxicity level, biodegradability, and immunogenic properties.^[4]

Recently bacterial cellulose (BC), produced by microbial cells and the cell-free enzyme systems,^[5,6] has been receiving immense consideration as the packaging material, in addition to its direct use as the food material in the form of 'nata de

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coco'.^[7] BC possesses unique features such as biocompatibility, biodegradability, purity, fibrous nature, flexibility, moldability, optical transparency, and low density.^[8] Owing to these features, BC finds several other bio-applications in areas like wound dressing,^[9,10] biosensing,^[11] environment remediation,^[12] bioprinting,^[13,14] drug delivery,^[15] synthetic organs,^[16] and others.^[17] However, due to lacking innate antimicrobial activity, BC is not directly used for many bio-applications, for instance, in smart packaging or active food packaging applications.^[18] Therefore composites of BC have been developed with a range of antimicrobials such as nanoparticles,^[19,20] clays,^[21] and some polymers^[22] to impart it antimicrobial activity against different microorganisms for its application as active food packaging material.^[23,24] Considering the fibrous and porous nature of the BC matrix, it allows the impregnation of a variety of materials of different shapes, sizes, and nature (solids and liquids).

To date, the potential of different antimicrobials such as bacteriocins, enzymes, organic acids, and different vegetable oils has been explored for imparting antibacterial activity to the packaging materials for providing protection to the food against the invading microorganisms, thus preventing their spoilage and increasing the shelf-life.^[25–28] However, the wide range of use of such materials in packaging applications has been limited by their high cost. To this end, the utilization of nanomaterials could be an alternative approach considering their low cost and high antimicrobial activities. For instance, silver nanoparticles (AgNPs) are considered the most effective antimicrobials against a broad spectrum of pathogens, including fungi, yeasts, bacteria, and viruses.^[29,30] A variety of AgNPs-based functional materials have been developed for various applications such as wound dressings,^[19] food packaging,^[31] antibacterial agents,^[32] separation and purification membranes,^[33] and protein analysis.^[34] Although the use of AgNPs as an antimicrobial agent, for instance, in food packaging, has been widely explored, their direct interaction with living tissues could cause toxicity if used in high concentrations and thus should be combined with other biocompatible material. For instance, the development of composites of AgNPs with cellulose, either through *in situ* reduction or *ex situ* impregnation, has not yet been evaluated as active and biocompatible food packaging materials.

Considering the prime need for safe, efficient, and edible food packaging materials of the low-cost and simple manufacturing process, acceptable water barrier features, and outstanding mechanical strength, the current study was aimed to develop antimicrobial Ag/BC films as bioactive and biocompatible food packaging materials. The developed Ag/BC nanocomposite film was characterized for its physico-chemical, mechanical, and thermal properties. The biological safety of the developed nanocomposite film was evaluated *in vitro*, while its antibacterial activity was evaluated against several bacterial and fungal strains. The developed film was tested for its food packaging application by analyzing the sensory features such as color, odor, dryness, and

contamination of tomatoes and oranges at different storage temperatures.

2. Experimental section

2.1 Materials

The chemical substances, including microbiological media, tryptone, disodium phosphate, glucose, citric acid, yeast extract, HCl, NaOH, AgNO₃, and NaCl were supplied by Sigma-Aldrich (St. Louis, MO, USA). Other chemical reagents, including Na₂HPO₄, (NH₄)₂SO₄, MgSO₄, and sucrose were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). The BC-producing *Gluconacetobacter xylinus* (ATCC53582) was obtained from the General Collection Center for Microbiological Culture in China (Beijing, China).

2.2 BC production and purification

BC was produced by *G. xylinus* (ATCC53582), as reported previously.^[35] Briefly, a few colonies of *G. xylinus* from agar plates were inoculated into liquid Hestrin-Scharmm (HS) medium (pH 5) containing 20 g/L glucose, 5 g/L yeast extract, 5 g/L peptone, 1.5 g/L citric acid, and 3.4 g/L disodium phosphate and incubated at 30 °C under shaking at 150 rpm for 24 h. Thereafter, 5% (v/v) pre-culture of *G. xylinus* was inoculated into 1 L sterilized HS medium along with 1% (v/v) absolute ethanol and incubated statically at 30 °C for 7–10 days. The BC hydrogel produced at the air-medium interface was harvested and washed in running water. Thereafter, the BC sheet was treated with 0.3 N NaOH for 12 h and autoclaved for 15 min at 121 °C and 15 psi to kill any live bacterial cells. Finally, the BC sheet was washed several times with distilled water until a neutral pH was achieved and stored at 4 °C for further use.

2.3 Ex situ synthesis of BC/Ag nanocomposite

The BC/Ag nanocomposite was prepared *ex situ* through reduction of silver nitrate (AgNO₃). Briefly, the BC sheets were dipped in 100 mL of 1 mmol/L AgNO₃ and stirred for 1 min, followed by the addition of 100 mL of 1 mmol/L NaCl and allowed to stir at 250 rpm for 1 min. Thereafter, the BC/Ag nanocomposite was washed three times with distilled water. This process was repeated at least 10 times to allow maximum reduction of AgNO₃ and impregnation of Ag nanoparticles into the BC matrix. Finally, the BC/Ag nanocomposite was washed with distilled water for 10 min to remove excess solutions.

2.4 Characterization of BC/Ag nanocomposite

The developed BC/Ag nanocomposite film was characterized for various properties. For scanning electron microscopy (SEM) analysis, the samples were fixed on double-sided tape on aluminum stubs and then coated with gold (40–50 nm). The chemical structure of pristine BC and BC/Ag nanocomposite films was investigated through Fourier transform infrared (FTIR) spectroscopy (VERTEX 70, Bruker, Germany). The

spectra for both samples were recorded in the spectral range of 500 to 4000 cm^{-1} at a resolution of 0.5 cm^{-1} . The X-ray diffraction (XRD) patterns of both films were recorded using an X-ray diffractometer (VG, Multilab 2000, USA) in the scanning angle between 0 to 90°. The crystallinities of pristine BC and BC/Ag nanocomposite were calculated from the relative integrated areas of crystalline and the amorphous peaks by using Eq. (1).

$$X_c = \frac{A_{cr}}{(A_{cr} + A_{am})} \times 100 \quad (1)$$

Where A_e and A_{am} are the integrated areas of the crystalline and amorphous peaks.

The mechanical properties, including the tensile strength and elongation at break, of pristine BC and BC/Ag nanocomposite films of dimensions 1.5×10 cm were determined by using 50 kg (Transcell Scale Co. Ltd., USA) compressive testing device SANS CMT4000 (MTS Industrial System Co., Ltd., China). In addition to an initial separation of 5 cm, the films were suspended and dismantled by 25 mm/min. The tensile strength was determined by dividing the average resting force (reading from the tool or chart) by a transverse film region ($\text{N/m}^2 = \text{Pascal}$). The percentage extension at rest was based on a longer duration relative to the original length of the film. The elongation at break was calculated by using Eq. (2).

$$\text{Elongation at break (\%)} = \frac{L - L_0}{L_0} \times 100 \quad (2)$$

Thermogravimetric analysis (TGA) of pristine BC and BC/Ag nanocomposite was performed by using a thermogravimetric/differential analyzer (Q50 Thermo balance, USA). Thermograms were obtained in the temperature range of 30–600°C under a nitrogen atmosphere with a temperature increase of 20°C/min.

2.5 Solubility and moisture content

The moisture content of pristine BC and BC/Ag nanocomposite films was determined according to a previously reported study.^[36] Briefly, the films were cut into 20 mm $\times$ 20 mm pieces, dried at 110 °C for 24 h, and weighed for the initial dry weight. After that, the dried films were immersed in 20 mL distilled water and kept at 60 °C for 3 h. Thereafter, the film fragments were filtered over a nylon cloth and washed with 10 mL distilled water, and dried at 110 °C for 24 h, and the dried weight of the film was determined again. The experiment was performed in triplicate. Finally, the water solubility (WS) was calculated from Eq. (3) as the ratio of original dry weight (W_o) and the dry weight after immersion in water and drying (W_f).

$$\text{WS(\%)} = \frac{W_o - W_f}{W_o} \times 100 \quad (3)$$

Similarly, the water content (WC) of the films was determined from Eq. (4) as the ratio of film weight before drying (W_b) and after drying (W_o).

$$\text{WC(\%)} = \frac{W_b - W_o}{W_b} \times 100 \quad (4)$$

2.6 Antibacterial activity

The antibacterial activity of BC/Ag nanocomposite was determined against *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *Trichosporone sp.* via disc diffusion method, as reported previously.^[37] Briefly, all microbial strains were cultured on a nutrient agar medium or yeast-peptone-dextrose-agar (YPDA) medium. Briefly, the samples were cut into 8 mm diameter discs and UV-sterilized. Thereafter, the dried discs were placed on top of the agar culture plates of selected microorganisms and incubated for 24 h at 37 °C. Finally, the diameters of the inhibition zones were measured. Pristine BC and a suspension of Ag nanoparticles were used as the negative and the positive controls, respectively.

2.7 Toxicity analysis

The toxicity of BC/Ag nanocomposite against NIH-3T3 (mouse embryonic fibroblast) cells was determined via MTT assay. Briefly, NIH-3T3 cells were cultured in flasks in high glucose culture flasks (4.5 g/L) containing L-glutamine and pyruvate (110 g/L), DMEM, 10% FBS supplement (GIPCO, USA), and 1% penicillin/streptomycin, and incubated in 5% CO_2 at 37 °C. The culture medium was changed every day until confluent culture. For toxicity analysis, the BC/Ag nanocomposite films were placed in the 96-well microplate, seeded with 1×10^4 cells/well, and incubated in a 5% CO_2 incubator. After incubation for 24 to 48 h, the samples were washed three times with PBS and transferred into a fresh DMEM growth medium containing MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide, 5 mg/mL) reagent at 10:1. The samples were incubated again at 37 °C for 4 h. Thereafter, the medium was removed, followed by the addition of formazan and 150 mL of DMSO (dimethyl sulfoxide). Finally, the absorption was measured at 570 nm by using a multi-scan spectrophotometer (Tecan, Infinite F50, Germany). The cells cultured in medium only were used for reference, and their viability was considered 100%.

2.8 Fruits packaging

The packaging ability of BC/Ag nanocomposite films for oranges and tomatoes was determined by the coating method, as reported in our previous study.^[22] Fresh oranges and tomatoes were obtained from a local supplier (Wuhan, China) and sterilized for 2 min at the commercial maturity with 200 ppm NaClO solution and allowed to air dry. Thereafter, the oranges and tomatoes were coated with BC/Ag thin film. The samples were monitored regularly in sterile conditions to ensure that these were not adversely affected by the storage conditions. All samples were divided into four groups according to different temperatures: 6 °C (refrigerator), 30 °C and 40 °C (incubator), and 20–25 °C (room temperature) group. Each treatment group was comprised of three different samples, including the uncoated (negative control), BC-coated (reference), and BC/Ag-coated fruit samples, and each sample was used in triplicate. During incubation, the freshness of fruits was assessed for sensory features such as odor, color, dryness, and contamination, as reported in our previous

study.^[22] Briefly, the sensory features of fruits were assessed by a panel of ten judges, who are expert food biotechnologists, according to a scale of 1–10 with 1–2 = very poor, 3–4 = poor, 5–6 = fair, 7–8 = good, and 9–10 = excellent. A score of 5 was used as the cutoff value for product acceptability.

2.9 Statistical analysis

All experiments were performed in triplicate, and the results are given as the mean $\pm$ standard deviation of the mean. The Student's *t*-test was performed using the SPSS 22.0 software (IBM, Armonk, USA) for comparing the mean values for the control and the treatment groups. The difference was statistically significant at $*P < 0.05$ or $**P < 0.01$.

3. Results and discussion

3.1 Appearance, moisture content, and water solubility of BC/Ag nanocomposite film

BC possesses an ultrafine and well-organized nanofibrillar network structure that is capable of retaining a large amount of water and is biodegradable and non-toxic in nature. It is produced through simple microbial fermentation and is easily purified. Moreover, it can be molded into any shape during microbial biosynthesis. All these features make BC an appealing biopolymer for various bio-applications. Pristine BC is insoluble in water and common organic solvents; nevertheless, it can be dissolved in ionic liquids.^[38,39] In the present study, a precipitate of silver chloride was selected for composite synthesis with BC owing to its strong antibacterial activity and low toxicity compared to the silver ions. Furthermore, because silver chloride is poorly water-soluble, we expect to obtain a strongly bound silver chloride on BC film and avoid leaching the aqueous environment. A naked-eye observation revealed that BC was transformed from the transparent milky white into an opaque white membrane upon interaction with silver chloride, indicating that the macroscopic shape of the membrane was altered to some degree upon impregnation of AgNPs.^[40]

The results of moisture content and water solubility of pristine BC and BC/Ag nanocomposite films are shown in Table 1. The moisture content of pristine BC was decreased from $96.7 \pm 7.69\%$ to $95.1 \pm 11.53\%$ upon addition of AgNPs into its matrix, indicating no significant change. This result is in agreement with a previous study.^[40] Similarly, the water

solubility of BC was slightly increased from $3.20 \pm 2.78\%$ to $4.60 \pm 2.51\%$ upon the addition of AgNPs. This low solubility of BC could be attributed to the hydrophilic and insoluble nature of cellulose in water and common solvents due to its long stiff chains and the presence of free hydroxyl (OH) groups that form strong intra- and intermolecular hydrogen bonds between the chains.^[41,42] The results of moisture content and water solubility of BC/Ag nanocomposite are comparable with chitosan, a similar biopolymer to cellulose.^[43] A study showed that the addition of nanoparticles into chitosan film slightly decreased the moisture content,^[36] which could be attributed to the compactness of cellulose or chitosan network structure.

Table 1. Water solubility and moisture content of pristine BC and BC/Ag nanocomposite films.

Film	Moisture content – WC (%)	Water solubility - WS (%)
BC	96.7 ± 7.69	3.20 ± 2.78
BC/Ag	95.1 ± 11.53	4.60 ± 2.51

3.2 Morphology of BC/Ag nanocomposite film

SEM micrograph of pristine BC (Fig. 1A) shows a fibrous and porous morphology, which provides an ideal environment for the impregnation of nanoparticles and other materials. SEM observation of the surface of BC/Ag nanocomposite film (Figs. 1B and C) confirms the formation of AgNPs through the reduction of AgNO₃ by NaCl on the BC membrane. In the composite film, AgNPs appear as the illuminating particles in the detection mode due to their high atomic weight. It appears that the nanoparticles are several tens of nanometers in size. The nanometric particles often show up smaller AgCl nanoparticles and larger agglomerates, which indicate that new nanoparticles are formed after each dipping phase. During the early soaking phase, the formation of large size particles was initiated, which grew in size with increasing dipping processes. The reduction of AgNO₃ by NaCl resulted in the adsorption of a large amount of Ag ions between the cellulose fibers, leading to massive and widely distributed particle sizes. Several earlier studies have shown that the micro-structural surface of BC-based composites containing Ag demonstrates a microstructure.^[40,42,44]

Fig. 1 SEM micrographs of the surface morphology of (A) pristine BC and (B, C) BC/Ag nanocomposite film at different resolutions.

3.3 Mechanical properties of BC/Ag film

Tensile strength refers to the resistance of a substance to rupture when subjected to tension, denoted as stress/strain,^[45] while elongation at break refers to the extension of a film length from the original length to the breakpoint.^[46] The mechanical properties, including the tensile strength and elongation at break, of pristine BC and BC/Ag nanocomposite films were determined, and the results are shown in Fig. 2 and Table 2. When compared to the pristine BC film, the addition of AgNPs into the BC matrix increased the tensile strength from 17.02 ± 1.18 to 25.76 ± 3.21 MPa and elongation at break from $4.77 \pm 0.56\%$ to $6.18 \pm 0.98\%$, which is consistent with a previous study where the addition of AgNPs into cellulose matrix improved the plasticizing behavior and flexibility of cellulose/AgNPs composite material.^[47] An increment in mechanical properties of BC is also reported with other materials where the addition of a reinforcement material contributed to increasing the mechanical strength of BC.^[48–50] A study reported an increment in tensile strength of the composite when nanoparticles were incorporated into the chitosan-based films.^[43]

Fig. 2 Tensile strength (stress-strain) curves of pristine BC and BC/Ag nanocomposite films.

3.4 FTIR analysis of BC/Ag films

FTIR spectroscopy was carried out to confirm the presence of AgNPs in the BC matrix and determine the nature of the interaction between the cellulose fibers and AgNPs. Fig. 3 shows the purity of BC and successful synthesis of BC/Ag nanocomposite. FTIR spectrum of pure BC reveals the typical cellulose peaks pattern,^[41,51] confirming the purity of BC synthesized by *G. xylinum*, as well as the efficacy of post-

synthesis treatment with NaOH and repeated washing with distilled water. The characteristic bands of cellulose appeared at 3400 cm^{-1} , 2850 cm^{-1} , 1600 and 1300 cm^{-1} , and 1440 cm^{-1} for O-H stretching, C-H stretching, asymmetrical CH_2 stretching, O-H deformation, and CH_2 deformation, respectively.^[51] The major peaks associated with AgNPs and BC appeared as O-H stretching band at 3400 cm^{-1} , C-H stretching band at 2850 cm^{-1} , carbonyl stretching band at 1600 cm^{-1} , C-H vibration band at 1400 cm^{-1} , and C-H vibration band 840 cm^{-1} . The BC/Ag nanocomposite film did not show apparent variations with high absorption peaks compared to the pure BC. Therefore, it can be assumed that the impregnation of AgNPs into the BC matrix did not form new chemical bonds and did not alter the molecular and intermolecular interaction in the polymer matrix. These data are in accordance with previous studies.^[44,52,53]

Fig. 3 FTIR spectra of pristine BC and BC/Ag nanocomposite films.

3.5 XRD analysis of BC/Ag nanocomposite film

XRD analysis is used in materials research to examine the crystalline structure, the proportion of the crystalline areas to the amorphous regions, crystal size, and the organization patterns of crystals.^[54] In the present study, XRD analysis was carried out to investigate the microstructural changes that may occur in the BC matrix due to the adsorption and penetration of AgNPs (Fig. 4). XRD pattern of pristine BC showed two major peaks centered at $2\theta = 14.5^\circ$ and 22.8° along with a small shoulder peak at $2\theta = 17.0^\circ$. This peak pattern is in accordance with cellulose I polymorphic structure, as reported

Table 2. Mechanical properties of pristine BC and BC/Ag film.

Samples	Tensile strength (MPa)	Elongation at break (%)	Tensile elastic modulus Et (MPa)
BC	17.02 ± 1.18	4.77 ± 0.56	457 ± 32
BC/Ag	25.76 ± 3.21	6.18 ± 0.98	507.5 ± 39

in previous studies.^[35,41,55] In contrast, the XRD pattern of BC/Ag nanocomposite film showed the characteristic peaks of cellulose, however of low intensity, along with small peaks at $2\theta = 26^\circ$, 32° , 46° , and 54° , corresponding to the respective crystalline planes (111), (200), (220), and (311) of AgCl. These observations are in accordance with previous reports of developing BC-based composites with Ag^[40,56,57] or Ag-ZnO hybrid.^[54,58] The decreased intensity of cellulose peaks was further verified quantitatively by determining the crystallinity of pristine BC and BC/Ag nanocomposite. The relative crystallinities of pristine BC and BC/Ag nanocomposite films were found to be 69.51% and 52.77%, respectively. The decreased crystallinity of the BC/Ag nanocomposite films was clearly demonstrated by the low intensity cellulose peaks and small peaks due to the presence of AgNPs in the composite. It should be noted that the calculated values of crystallinity might deviate to some extent from the actual crystallinity values of the samples due to the presence of small peaks, which raise the background and increase the amorphous and total area.^[59]

Fig. 4 XRD pattern of pristine BC and BC/Ag nanocomposite films.

3.6 Thermogravimetric analysis of BC and BC/Ag nanocomposite films

All compounds have their unique thermal-degradation temperatures. A TGA thermogram not only provides information about the heat tolerance capabilities of different materials but also guarantees their purity. In the present study, the information on the thermal decomposition behavior and surface adsorbed water of the pristine BC and BC/Ag nanocomposite films are shown in Fig. 5. According to a previous study, the BC film possesses two different degradation regions, mainly associated with weight loss due to dehydration and cellulose decomposition.^[35] The natural nanofibers demonstrate excellent thermal stability, which further increases with the addition of thermally stable materials such as nanoparticles.^[60] The initial weight loss of

2% and 7% at 90°C and 120°C for BC and BC/Ag nanocomposite, respectively, was due to the evaporation of surface adsorbed water. Although freeze-dried BC was used for TGA analysis, the hydrophilic nature of BC allows the absorption of moisture when operated in the open air for analysis. Moreover, the removal of water molecules present in the interlayers also contributes to the observed weight loss.^[61] The second degradation phase of pristine BC started above 210°C and lasted until the cellulose chains were completely degraded above 350°C . In contrast, the second phase of weight loss for BC/Ag nanocomposite film started at 250°C and lasted at 350°C . During this stage, the degradation of the cellulose skeleton resulted in 75% and 55% weight loss of BC and BC/Ag nanocomposite, respectively. A literature analysis shows that the main skeleton of cellulose degrades above 200°C , and the complete degradation takes place above 300°C .^[41] From this point forward, the TGA thermogram of pristine BC shows its thermal decay between 320°C to 450°C in two stages. The residual 21% weight, comprised of carbon and AgNPs, was obtained at above 600°C .^[62] The effect of heat degradation supports not only the well-ordered structure of BC fibrillation but also its purity, as indicated by the absence of additional degradation areas in TGA thermograms.^[63,64] The TGA thermograms of BC/Ag nanocomposite film showed enhanced thermal stability, which could be helpful for the sterilization of packaging materials. The findings of this study are consistent with previous research, indicating an improvement in the thermal properties of chitosan nanocomposite with silver.^[43] The thermal behavior of polymer-based nanocomposites is affected by the dispersion of nanoparticles within the polymer matrix, hydrogen bonding, and interactions between the polymer chains and nanoparticles.^[65] Thus, the uniformly dispersed AgNPs increased the thermal stability of BC film, indicating that nanoparticles can significantly improve the thermal stability and thermal properties of films.

Fig. 5 TGA curves of pristine BC and BC/Ag nanocomposite films.

3.7 Antibacterial activity of BC/Ag films

The AgNPs and Ag⁺ ions are known to possess strong antimicrobial activity and high inhibitory effects towards different microbes, such as *Escherichia coli*. Earlier studies have demonstrated the successful development of BC-based antimicrobials with a variety of nanoparticles such as Pt, Pd Cu, and TiO₂.^[37,38,60] In the present study, the antimicrobial activity of pristine BC and BC/Ag nanocomposite film was evaluated against five pathogens, including three bacterial strains named *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli*, and two fungal species named *Candida albicans* and *Trichosporon sp.*, along with negative control (pure BC film) and positive control (*i.e.*, AgCl loaded on filter paper), and the results are shown in Fig. 6. The selected microbial strains for the determination of the antimicrobial activity of BC/Ag nanocomposite are generally associated with human health and are mainly responsible for food contamination. The antimicrobial activity evaluation through the disc diffusion method showed that the BC/Ag nanocomposite discs produced inhibition zones of 0.08 cm, 0.065 cm, 0.17 cm, 0.08 cm, and 0.162 cm for *Trichosporon sp.*, *C. albicans*, *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively. These values are significantly lower than the positive control (**P* < 0.05) but higher than the negative control (***P* < 0.01). In contrast, the AgCl-loaded filter paper (*i.e.*, positive control) produced inhibition zones of 0.1 cm, 0.1 cm, 0.15 cm, 0.13 cm, and 0.175 cm against the same set of microbial strains. As expected, the pristine BC (*i.e.*, negative control) did not produce any inhibition zone against the selected microbial strains, indicating that the antibacterial activity of BC/Ag nanocomposite was solely due to the AgNPs

and that their impregnation into the BC matrix did not significantly alter their bactericidal activity. Moreover, the developed BC/Ag nanocomposite film is effective both against the Gram-positive and Gram-negative bacterial and fungal species and thus can be an effective food packaging material. These results are in accordance with previous studies where AgNPs have been used in the development of efficient antimicrobial films for food packaging application to impart antimicrobial activities to the films and enhance the shelf-life of food.^[30,66,67]

3.8 Biocompatibility testing of BC/Ag nanocomposite film

A polymer used for the development of edible packaging films must be biocompatible and non-toxic in nature. The nature of BC as a non-toxic and biocompatible polymer has been extensively evaluated both *in vitro* and *in vivo* for various bio-applications.^[68,69] In the present study, the cytotoxicity of BC/Ag nanocomposite film was evaluated against NIH-3T3 fibroblasts through MTT assay, and the results are shown in Fig. 7. The viabilities of NIH-3T3 fibroblasts on pure BC and BC/Ag nanocomposite films after 1 day were 67% and 63%, respectively, indicating no cytotoxic effect of the film towards cells (Fig. 7). With continuous incubation to day 3, the viability increased and reached 74% and 66% on BC and BC/Ag nanocomposite films. However, with further incubation to day 5, the viability of cells showed a decreasing trend and was found to be 61% and 57% on BC and BC/Ag nanocomposite films, respectively. This decreased cell viability on day 5 could be due to the depletion of nutrients in the growth medium. These results show acceptable cell viability both on BC alone and in the form of composite with

Fig. 6 Antimicrobial activity of BC/Ag nanocomposite film (test film), Ag (positive control), and BC only (negative control) against (A) *E. coli*, (B) *P. aeruginosa*, (C) *S. aureus*, (D) *C. Albicans*, and (E) *Trichosporon sp.* (F) Diameter (cm) of inhibition zone.

Fig. 7 Viability of NIH-3T3 cells cultured in pristine BC and BC/Ag nanocomposite films in 96-well plate. Absorption was recorded at 570 nm for all samples, * $p < 0.05$.

AgNPs. Although the cell viability was slightly lower on BC/Ag nanocomposites relative to the pristine BC (** $P < 0.01$), the cell survival on the latter was more than 90% compared to the control, indicating the acceptable cytotoxicity of AgNPs towards the NIH-3T3 fibroblasts. These results are in accordance with a previous study where the BC/Ag nanocomposite supported the growth of NIH-3T3 fibroblasts with an acceptable toxicity level.^[19] According to the findings of a study, Ag nanoparticles are generally non-toxic towards mammalian cells and only show toxicity due to their small size and volatile characteristics when used at higher concentrations.^[70]

3.9 Real food packaging under different storage conditions with BC/Ag nanocomposite film

The biofilm-based packaging has attained increasing attention for antimicrobial food coating, which can be used to extend the shelf-life of food products. The potential of AgNPs as an antimicrobial agent in the development of active food packaging materials has already been evaluated.^[30,66,67] A literature review shows that different forms of silver-based materials have been combined with a variety of materials for the packaging of different food products, indicating their usefulness in packaging application (Table 3). In the present study, the antimicrobial packaging potential of BC/Ag

Table 3. Food application of AgNPs with a variety of polymers

Biopolymeric matrix	Additives	Food Application	Reference
Alginate	Silver nitrate	Shiitake mushrooms	[71]
Linear low-density polyethylene	Cinnamon oil and silver-copper	Chicken meat	[72]
Pullulan	AgNPs and oils	Meat industry	[73]
Chitosan/BC	AgNPs	Food packaging	[43]
Low-density polyethylene	AgNPs	Orange juice	[74]
Low-density polyethylene	AgNPs	Barberry	[74]
Ethylene vinyl alcohol copolymers	AgNPs	Chicken	[75]
Polyethylene	AgNPs	Fresh apples	[76]
Hydroxypropyl methylcellulose	AgNPs	Food packaging	[77]
Poly(lactide (PLA)/nano-TiO ₂ and PLA/nano-TiO ₂	nano-Ag	Food packaging	[78]
cellulose fibers	silver nitrate	Fresh-cut melon	[30]
Polymer nanocomposites	Silver nitrate	Food containers	[79]
Pullulan	AgNPs	Turkey deli meat	[80]
Sodium alginate	AgNPs	Pears, carrots	[81]
Calcium alginate	Ag-montmorillonite NPs	Fresh-cut carrots	[51]
Bacterial cellulose	AgNPs	Orange and tomato	Present study

nanocomposite film was evaluated by coating the fruits, including oranges and tomatoes, and stored under four different storage temperatures: refrigerator (6 °C), room temperature (20 °C to 25 °C), and in high-temperature incubators at 30 °C and 40 °C.

Fig. 8 shows the qualitative results of different orange-coated samples, including the control (uncoated), BC-coated (designated as Film-0), and BC/Ag-coated (designated as Film-1), stored at various temperatures. The photographs show that the Film-1-coated oranges stored at 6 °C for up to 63 days showed no color or odor change, whereas Film-0 showed a light drought after 21 days and continued to increase until 63 days (Fig. 8A). The control, on the other hand, showed a moderate drought under the same storage conditions. Both the control and Film-0 oranges showed comparatively high drought after 14 days at room temperature (Fig. 8B), whereas film-1-coated oranges remained relatively fresh and preserved their quality after 28 days. In comparison to the Film-1 after 28 days at 30 °C (Fig. 8C) and 14 days at 40 °C (Fig. 8D), both the control and Film-0 showed brown spots with a drought-like appearance after 7 days at 30 °C (Fig. 8C) and 40 °C (Fig. 8D). Furthermore, while the quality of some sensory features, such as odor and color, differed between control and the test samples, some sensory features, such as odor and color, were comparable. On the other hand, Fig. 10 depicts the qualitative

results of tomato packaging with different samples incubated for different time intervals at different temperatures. Film-1 gave tomatoes high storage stability during the extended incubation at 6 °C (Fig. 10A), room temperature of 20 to 25 °C (Fig. 10B), 30 °C (Fig. 10C), and 40 °C (Fig. 10D). The control and Film-0 showed medium rot after 4 weeks at 6 °C, but the tomatoes began to spoil after one week at 30 °C, 40 °C, and room temperature.

Additionally, Fig. 9 and Table 4 demonstrate the quantitative analysis of findings for sensory characteristics such as odor and color of oranges according to a predetermined scale (1–2 = extremely bad, 3–4 = poor, 5–6 = fair, 7–8 = good, and 9–10 = excellent) and the value=5 represent the minimum acceptable value. The results show that at all treatment temperatures, except 6°C, the Film-1 showed a higher value while the control and Film-0 demonstrated lower values than the cutoff value. Additionally, the study of overall acceptability against time revealed that the value of Film-1-coated oranges remained greater than the cutoff value after 28 days at all treatment temperatures except 40°C. Also, the uncoated and Film-0-coated samples remained fresh at room temperature for only up to 14 days. These quantitative findings show that the coating of oranges with BC/Ag significantly increased their shelf-life up to 14 days. Literature review shows that nanomaterials have been widely

Fig. 8 The photographs of uncoated and coated oranges held at four different temperatures: (A) 6 °C, (B) room temperature around 20 to 25 °C, (C) 30 °C, and (D) 40 °C for 9 weeks. Control (orange without treatment), Film-0 (BC-coated oranges), and Film-1 (BC/Ag nanocomposite-coated oranges).

Fig. 9 Evaluation of acceptability of BC/Ag nanocomposite-coated oranges (Film-1), BC-coated oranges (Film-0), and uncoated oranges (control) kept at (A) 6 °C, (B) room temperature around 20 to 25 °C, (C) 30 °C, and (D) 40 °C for various time periods.

Table 4. Values of acceptability degree of BC/Ag-coated oranges and tomatoes (Film-1), BC-coated (Film-0), and uncoated (control) kept at different temperatures for various time periods.

Sample	Temperature (°C)	Oranges		Tomatoes	
		Minimum accepted value w.r.t sensory features	Minimum accepted value w.r.t time (days)	Minimum accepted value w.r.t sensory features	Minimum accepted value w.r.t time (days)
Control	6	5 ± 0.35	49	5 ± 0.45	21
	20-25	5 ± 0.4	21	8 ± 0.64	2
	30	6 ± 0.57	2	5 ± 0.4	7
	40	6 ± 0.54	2	9 ± 0.81	2
Film-0	6	5 ± 0.25	56	5 ± 0.5	28
	20-25	5 ± 0.47	14	7 ± 0.66	2
	30	7 ± 0.77	2	6 ± 0.54	7
	40	6 ± 0.54	7	9 ± 0.63	2
Film-1	6	8 ± 0.6	63	7 ± 0.56	28
	20-25	5 ± 0.25	42	6 ± 0.45	21
	30	6 ± 0.42	28	6 ± 0.42	14
	40	5 ± 0.4	14	8 ± 0.64	14

Sensory features: contamination, dryness, color, and odor; w.r.t: with respect to

Fig. 10 The photographs of uncoated and coated tomatoes held at four different temperatures: (A) 6 °C, (B) room temperature around 20 to 25 °C, (C) 30 °C (D), and 40 °C for 9 weeks. Control (tomato without treatment), Film-0 (BC-coated tomatoes), and Film-1 (BC/Ag nanocomposite film-coated).

studied as active polymeric nanocomposites for food packaging. For instance, AgNPs in particular have been used as a bactericidal agent for various bio-applications. In a study, AgNPs were impregnated into the matrix of hydroxypropyl methylcellulose for use as food packaging materials, where the disk diffusion experiments showed the efficient killing of bacteria.^[77] In another study, the antibacterial activity of cellulose/Ag nanohybrid material was tested during the

preservation of processed melon. The fresh-cut melon pieces were kept for 10 days at 4°C in a modified natural environment. The inclusion of silver-laden absorbent pads delayed the spoilage of melon, showing significantly low microbial counts after 10 days of storage.^[30]

Fig. 11 shows the findings of quantitative analysis of sensory characteristics such as odor and color of tomatoes according to the predetermined scale (1–2 = extremely bad, 3–

Fig. 11 Evaluation of acceptability of BC/Ag nanocomposite film-coated tomatoes (Film-1), BC-coated tomatoes (Film-0) and uncoated tomatoes (control), and kept at (A) 6 °C, (B) room temperature around 20 to 25 °C, (C) 30 °C, and (D) 40 °C for various time periods.

4 = poor, 5–6 = fair, 7–8 = good, and 9–10 = excellent), and 5 was set as the minimum acceptable value. At room temperature, the uncoated and Film-0-coated samples remained fresh just for a week, while the Film-1 coated samples remained fresh for 3 weeks. These results show improvement in the shelf-life of tomatoes when packaged with BC/Ag nanocomposite film. Additionally, the tomatoes covered with Film-1 showed a good level of acceptance at various storage temperatures. These findings are similar to the orange packaging with pristine BC or BC/Ag nanocomposite films. The packaging of tomatoes with BC/Ag nanocomposite film provided adequate packaging performance for prolonged storage, not only at 6 °C but also at room temperature and elevated temperatures of 30 °C and 40 °C for up to two weeks which is in accordance with previous studies.^[30,66]

Overall the findings of fruit coating with BC/Ag nanocomposite film indicate significantly improved protection of the texture of oranges and tomatoes from the effects of environmental factors and contribute to preserving their odor and color for an extended time.

4. Conclusions

The physicochemical characterization indicated improved features of BC upon impregnation with AgNPs. The addition of AgNPs to BC film enhanced its thermal and mechanical properties and decreased the crystallinity. The BC/Ag nanocomposite film showed better flexibility and good thermal degradation. Moreover, the BC/Ag film showed good antibacterial activity against Gram-positive and Gram-negative bacteria as well as fungi. In addition, the cell viability results showed the biocompatible and non-toxic nature of BC/Ag film towards NIH-3T3 fibroblasts. The coating of real food samples, including oranges and tomatoes, with BC/Ag nanocomposite enhanced their shelf-life due to its antimicrobial nature. The findings of this study provide a base for the development of biocompatible and edible fruit coating biomaterials, which could potentially replace the conventional non-degradable plastic-based coating and packaging materials.

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Conflict of interest

There are no conflicts to declare.

Supporting information

Not applicable.

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