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Antibacterial properties of films of cellulose composites with silver nanoparticles and antibiotics

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ABSTRACT

The present study describes production of bacterial cellulose composites with silver nanoparticles and antibiotics and compares their properties. Bacterial cellulose (BC) composites synthesized in the culture of the strain of acetic acid bacterium *Komagataeibacter xylinus* VKPM B-12068 with silver nanoparticles, BC/AgNps, were produced hydrothermally, under different $AgNO_3$ concentrations (0.0001, 0.001, and 0.01 M) in the reaction medium. The presence of silver in the BC/AgNp composites was confirmed by elemental analysis conducted using scanning electron microscopy with a system of X-ray spectral analysis. Analysis showed that the average atomic number of silver particles in composite samples depended on the concentration of $AgNO_3$: as $AgNO_3$ concentration in the reaction solution was increased, silver content in the composites increased from 0.044 to 0.37 mg/cm^2. BC composites with amikacin and ceftriaxone were prepared by immersing dry BC films in solutions containing different concentrations of the antibiotics. The surface structure and properties and physicochemical and mechanical characteristics of composites were investigated using SEM, DSC, X-ray analysis, the system for measuring water contact angles, and electromechanical tensile testing machine. The disk-diffusion method and the shake-flask culture method used in this study showed that all experimental composites had pronounced antibacterial activity against *E. coli, Ps. eruginosa, K. pneumoniae,* and *St. aureus*, and the BC/antibiotic composites were more active than BC/AgNp ones; *S. aureus* was the most susceptible to the effect of BC composites. No potential cytotoxicity was detected in any of the BC/AgNp composites in the NIH 3T3 mouse fibroblast cell culture, in contrast to the BC/antibiotic composites. These results suggest that BC composites constructed in the present study hold promise as dressings for managing wounds, including contaminated ones.

1. Introduction

Bacterial cellulose (BC) – a biopolymer synthesized by microorganisms – is a promising material for biomedical application. The chemical structure of BC is similar to that of plant-derived cellulose, but BC has unique physical, mechanical, and chemical properties. This material shows high biocompatibility, without being cytotoxic or causing any allergic reactions. Studies of BC suggest that this natural polymer can be useful for cellular and tissue engineering as material for constructing scaffolds and for reconstructive surgery as material for skin defect reconstruction and as a matrix for drug delivery [1,2]. Physical and mechanical properties of BC can be enhanced by preparing BC composites with various materials: chitosan [3], collagen [4], sodium alginate, gelatin, polyethylene glycol [5].

BC is not inherently antibacterial, but BC composites with chitosan and alginate inhibit growth of pathogenic microorganisms such as *E.coli, Candida albicans,* and *Staphylococcus aureus* [3,6,7]. Therefore, BC composite films can be considered for treating contaminated wounds. BS can be hybridized with metallic silver particles to produce an antibacterial and wound-healing formulation. Metallic silver and compounds thereof have a strong bactericidal effect, inhibiting development of a wide range of pathogenic microorganisms. Silver ions react with cell membrane protein thiol groups, affecting bacterial respiration and transport of substances through the cell membrane [8].

Production of BC composites with silver nanoparticles has been extensively discussed in the literature. Various approaches have been proposed of the in situ generation of Ag or Cu and other metals in cellulose matrix and cotton fabrics, by using different reducing agents and by hydrothermal methods, which are simpler to use and eco-friendly.

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Composites of silver nanoparticles and bacterial cellulose with high antimicrobial activity were prepared using Tollens' reaction [9] with silver reduced by polydopamine, which had been used as a medium for immersion of homogenized cellulose [10]. Other authors [11] used a more complex approach to producing silver nanoparticles: homogenized cellulose was first oxidized using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), which created carboxyl groups on the surface, and then Na^{+}-Ag^{+} exchange reaction was performed; the ions were reduced using $NaBH_4$ or sodium citrate. The silver particles produced by this method were of different sizes and effective against *Escherichia coli* and *Staphylococcus aureus*.

Different modifications of hydrothermal methods for production of nanosilver and other metals involve the use of bacterial cellulose and natural plant-derived agents as reductants. G. Yang et al. [12] used a hydrothermal method with cellulose employed as a reducing and stabilizing agent. By optimizing reaction parameters, the authors obtained narrow distribution of Ag particles, thus achieving an effective and durable antibacterial action. Composites of cellulose with silver sulfadiazine dispersed to nanosize were used as antimicrobial wound dressing [13], which facilitated the healing of model burns in rats. J. Wu et al. [14] reported a study in which model burns were treated with composites prepared by reducing Ag+ on the surface of cellulose fibers. The composites suppressed wound microflora and favored fibroblast attachment. Silver nanoparticles were produced in situ in cotton fabrics by the hydrothermal method at 80 °C [15]. The composites showed good antibacterial activity against Gram-negative and Gram-positive bacteria. A similar method was used to prepare a cotton composite with copper nanoparticles [16]. Cotton nanocomposite fabrics exhibited good antibacterial activity and were effective against both Gram-positive and Gram-negative bacteria. Hence, they can be used in medical applications: as wound dressings, surgical aprons, materials for hospital beds, etc. In another study [17], silver nanoparticles were produced in situ in cellulose matrix using *Ocimum* leaf extract as a reducing agent. Composite films showed good antibacterial activity and, thus, can be used for packaging and medical purposes. P. Sivaranjana et al. (2017) [18] reported producing cellulose nanocomposite films with in situ generated silver nanoparticles using *Cassia alata* leaf extract as a reducing agent. The nanocomposite cellulose/AgNP films had good tensile properties and showed antibacterial activity; therefore, they can be considered for medical applications as dressing materials.

A number of studies report successful uses of plant extracts as reducing agents for silver ions. In a study by L. Muthulakshmi et al. [19], copper nanoparticles (CuNPs) were generated in situ inside cellulose matrix using *Terminalia catappa* leaf extract as a reducing agent, and their properties were investigated. The composite films possessed sufficient tensile strength, and, thus, they can replace polymer packaging materials like polyethylene. Further, the cellulose/CuNP composite films exhibited good antibacterial activity against *E.coli*.

In another work [20], cellulose gel films with nanosilver were produced using a precooled mixture of 8 wt% lithium hydroxide and 15 wt% urea as a solvent and ethyl alcohol as a nonsolvent, with the *Terminus cattapa* leaf extract used as a reducing agent. Composite cellulose/AgNP films showed good antibacterial activity against *E. coli* and *Bacillus* sp. Hydrothermal synthesis was used to produce nanoparticles of other metals too. For instance, polyhedron-shaped hematite (a-Fe2O3) nanoparticles were successfully synthesized via a facile hydrothermal method by mixing FeCl3 and NH_4OH at high temperature [21].

Besides BC, recent publications demonstrated that cellulose nanocrystals and hydrogels could also be used as reductant and support for the synthesis of AgNPs. For example, Rui Xiong et al. [22] demonstrated a facile and environmentally friendly approach to prepare Fe_3O_4/Ag/nanofibrillated cellulose (NFC) nanocomposites, which enables tunability from highly porous, flexible aerogels to solid and stiff films. NFC acts as a biocompatible support for the magnetic silver nanoparticles and a reducing agent for the silver ions. The Fe_3O_4/Ag/NFC nanocomposite aerogel exhibited excellent catalytic properties for the reduction of 4-nitrophenol, and showed high antibacterial activity against the model microbe *S. aureus*.

Several studies showed advantages of using cellulose crystals as a reductant for producing nanosilver. Rui Xiong et al. [23] described production of well dispersed and stable silver nanostructures using cellulose nanocrystals without employing other reductants or dispersing agents. Moreover, the authors showed that morphology of silver nanostructures could be adjusted by changing $AgNO_3$ concentration and producing silver shaped as nanospheres or dendrites with different antibacterial activity (more pronounced in dendritic silver) against *Escherichia coli* and *Staphylococcus aureus*. Successful use of cellulose crystals for producing hybrid silver/biodegradable polymer nanocomposites as potential food packaging was reported by Yu et al. The authors produced nanocomposites consisting of biodegradable nanohydrates poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and cellulose/silver nanocrystals (CNC-Ag) with improved thermal, mechanical, and antibacterial properties [24]. The authors found that homogeneously dispersed CNC-Ag could serve as a factor improving the properties of the pristine polymer. In another study [25], the authors described fabrication of multifunctional cellulose nanocrystals/poly (lactic acid) nanocomposites with silver nanoparticles by spraying method. Deposited silver (Ag) nanoparticles and CNF effectively reinforced mechanical properties and antibacterial activity of polylactide as well as water vapor permeability of the composite film, which was important for the packaging material. In a study by W. Xu et al. [26], cellulose nanocrystals (CNCs) produced by hydrolysis of sulfuric acid were dispersed in polycarbonate (PC) in organic solution to prepare a composite. Due to the good dispersion of nanofillers in polymeric matrix, strong hydrogen bonds were formed between carbonyl groups of polycarbonate and hydroxyl groups of cellulose, which caused improvement of thermal and mechanical properties of the composite films.

A novel and straightforward synthetic strategy was developed to prepare silver nanoparticles-doped cellulose microgels (AgNPs/CMG) nanohybrids at room temperature [27]. The as-prepared AgNPs/CMG nanohybrids exhibited excellent catalytic performance in reduction of 4-nitrophenol and organic dyes. The simplicity, sustainability, and straightforwardness of this approach to prepare a highly efficient catalyst and functional membrane open up new possibilities for large-scale production and application of bioresources/noble metal nanohybrids in various fields.

Another approach to imparting antibacterial activity against pathogenic microflora to BC is to prepare BC composites with antibiotics. As a potential wound dressing for treating acute traumas, freeze-dried BC film was loaded with benzalkonium chloride – an antimicrobial agent of cationic surfactant type. Antimicrobial activity of the composite was observed for 24 h against *Staphylococcus aureus* and *Bacillus subtilis*, which were the major bacteria in the contaminated wound [28]. Composites of bacterial cellulose with tetracycline hydrochloride, BC-TCH, were produced and characterized by other authors [29,30]. The composites exhibited excellent antibacterial activity and good biocompatibility and enabled controlled release of the antibiotic.

Analysis of the literature suggests that production of nanocomposites based on bacterial cellulose is a promising and important subject of research. However, most of the studies describe production of BC composites with nanosilver and characterize them as dependent on the technique of production and ratios of components in the composites. Much less consideration has been given to the properties of BC composites with antibiotics.

Therefore, the purpose of this study was to prepare bacterial cellulose composites with silver nanoparticles and antibiotics and to compare their properties and antibacterial activity.