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Nanomaterials from bacterial cellulose for antimicrobial wound dressing

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Abstract. Bacterial nanocellulose (BNC) is widely used in biomedical applications. BNC has attracted increasing attention as a novel wound dressing material, but it has no antimicrobial activity. To get over this problem in the present study the BNC was saturated with antibiotic fusidic acid (FA). The subject of the experiment was BNC, produced by bacteria \textit{Gluconacetobacter sucrofermentans} B-11267. The resulting biocomposites have high antibiotic activity against \textit{Staphylococcus aureus} and can be used in medicine as a wound dressing. The structure of BNC was analyzed by atomic force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR).

1. Introduction

Cellulose is well known as one of the most abundant biodegradable materials in nature. It is traditionally extracted from plants but can also be produced by certain bacterial species by fermentation. Bacterial cellulose (BC) is produced mainly by microorganisms belonging to genera \textit{Gluconacetobacter} (renamed as \textit{Komagataeibacter}) \cite{1}. BC is chemically identical with plant cellulose but it is not contaminated such products as lignin, pectin, and hemicelluloses \cite{2}. BC has a unique reticulate network from thin fibers with diameter more than 100 times smaller than that of plant-derived fibers \cite{2-4}. BC has better mechanical properties, higher water-holding capacity, higher crystallinity, higher porosity, larger surface area, ultrafine network structure and biocompatibility as compared with plant cellulose \cite{3-7}.

This unique nanostructured matrix is being widely explored for various medical and nonmedical applications. BNC is used extensively in many fields, including biomedical materials \cite{1, 8-10}, food industry \cite{11}, acoustic diaphragm, functional paper \cite{12}, drug delivery \cite{13}, tissue engineering \cite{14}, nanostructured biomaterials and biocomposites \cite{15-17}.

BC has many characteristics of an ideal wound dressing. It is known that BC reduce pain and accelerate granulation, ensuring proper wound healing \cite{18, 19}. Further, BC helps in creation of a moist environment at the wound area, absorption of exudates. Thereby BC is considered as as physical barrier between the wound and the surrounding environment, preventing microbial infections. But BNC in its native state does not exhibit antimicrobial effects. There are several successful attempts to
impair antimicrobial properties to BC. Bacterial cellulose-silver nanocomposites were successfully prepared and they exhibited excellent antibacterial activity [20-22]. The antibacterial composite based on BC was successfully prepared by in-situ synthesis of SiO$_2$ coated Cu nanoparticles (Cu@SiO$_2$/BC) [23]. The synthesis of BNC/chitosan composites with high mechanical reliability and antibacterial activity was reported [24]. The antiseptics povidone-iodine (PI) and polihexanide (PHMB) were loaded into BNC with the idea to supplement the superior material properties of the nanostructured biopolymer as a wound dressing with antimicrobial activity [25]. Antibiotics are used in combination with BC for the preparation of composite membranes with antibacterial activity. For example, an antibiotic drug tetracycline hydrochloride (TCH)-loaded bacterial cellulose composite membranes were prepared [26].

The aim of this study was to prepare antibiotic fusidic acid loaded BNC biocomposites and evaluate their antibacterial activities.

2. Materials and methods

2.1. BNC Preparation

BNC was prepared in a static culture medium by *Gluconacetobacter sucrofermentans* B-11267, which was isolated from Kombucha tea and identified by sequencing the amplified product of 16S rRNA [27]. For BNC production HS medium was used (g/L): glucose (20), peptone (5), yeast extract (5), citric acid (1.15), and disodium hydrogen phosphate (2.7), pH 6.0. Culture media was autoclaved at 121°C 20 min. The media was inoculated with 10 % (v/v) inoculum. To prepare the inoculum, *G. sucrofermentans* B-11267 from an agar plate was transferred aseptically into a 250 ml Erlenmeyer flask containing 100 ml of culture medium and incubated on a shaker incubator (Model ES-20/60, BIOSAN, Latvia) at 28 °C for 24 h at 250 g. BNC was produced in static conditions at 28 °C for 5 days. After incubation, BNC was collected, washed thoroughly with de-ionized water to remove medium components, and treated with 4 % (w/v) sodium hydroxide solution at 80 °C for 1 h to eliminate bacterial cells. Further, BNC was rinsed extensively with 6 % acetic acid and then with de-ionized water until pH became neutral. The purified BNC was dried to constant weight at 60 °C and then weighed.

2.2. Production of BNC/FA composites

BNC films were cut into round shapes with 10 mm diameter and immersed in 10 mL aqueous sodium fusidate solution with concentrations of 0.1, 0.2, 0.3 and 0.4 g/L for 1-24 h. Then they were dried at 60 °C for 24 h. The final composite films were named as BNC$_{0.1}$, BNC$_{0.2}$, BNC$_{0.3}$ and BNC$_{0.4}$, respectively.

2.3. Characterization

The surface morphology of BNC was studied by contact atomic force microscopy (AFM) using an SPM 9600 (Shimadzu, Japan).

FTIR spectra of BNC were obtained using a Fourier transform infrared spectrometer IRPrestige-21 (Shimadzu, Japan).

2.4. Antibacterial activity

The antibacterial activities of BNC/FA composites were investigated by disk diffusion method against *Staphylococcus aureus* 209 P. Lawns of test bacteria (about $1 \times 10^5$ CFU/plate) were prepared on medium N1 GRM for bacteria cultivation. The sterilized samples were then carefully placed upon the lawns and sodium fusidate solution with concentrations of 0.1, 0.2, 0.3 and 0.4 g/L was used as control. The plates were placed in a 37°C incubator for 24 h. Then inhibitory action of tested samples on the growth of the bacteria was determined by measuring diameter of inhibition zone.

3. Results and discussion

3.1. Morphology of BNC
The macrostructure morphology of BNC varied depending on the different culture methods. In stationary culture, a gelatinous cellulose film is formed on the air/liquid interface of the medium. In agitated culture, cellulose is synthesized in deep medium in the form of fibrous suspensions, pellets, irregular masses [28]. When cultivating bacteria *G. sucrofermentans* B-11267 in static conditions in HS growing medium for 5 days on the surface of the medium a BC gel film was formed. After treatment a gel film became colourless and transparent (figure 1).

![Figure 1. BC gel film.](image1.png)

To study the microscopic details of the biopolymer, atomic force microscopy (AFM) was used. Figure 2 shows the micro-morphology of BNC that exhibited a nanoporous three-dimensional network structure with a random arrangement of ribbon-shaped fibrils. The thickness of BNC fibrils formed on standard HS medium averaged 50-90 nm.

![Figure 2. AFM image of the BNC.](image2.png)

### 3.2. FTIR spectroscopy

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Figure 3 displays the FTIR spectra of BNC, FA and BNC \(_{0.4}\) composite membranes. FTIR spectrum of BNC (figure 3a) was typical for this polymer. At the FTIR spectrum of the BNC the absorption at the area 3200-3500 cm\(^{-1}\) corresponds to the valence vibration of hydrogen bonded OH groups [26]. In the C-O stretching vibration region the peaks at 1164, and 1061 cm\(^{-1}\) correspond to the C-O-C asymmetric stretching and the C-C, C-OH, C-H ring and side group vibrations, respectively. The infrared spectrum of pure FA exhibited three characteristic bands at 3420, 1725, 1260 cm\(^{-1}\) (figure 3c). Absorption at the 3420, 1725 cm\(^{-1}\) were caused by valence vibration of hydrogen bonded OH groups and carboxyl groups of the FA. In the case of BNC \(_{0.4}\) composite the FTIR spectrum had typical bands for BNC and FA (figure 3b). The band in the 3600-3100 cm\(^{-1}\) region became broader and more extensive. Absorption at this region can give considerable information concerning the hydrogen bonds. Probably broadening of this peak was caused by forming additional intermolecular hydrogen bonds between BNC and FA. At the FTIR spectrum of the BNC \(_{0.4}\) composite the bands 1725 and 1260 cm\(^{-1}\), that are typical for FA can be detected. Presence of these peaks at the FTIR spectrum of the BNC \(_{0.4}\) indicates presence of the FA in the obtained composite.

![Figure 3. FTIR spectra of BNC (a); BNC \(_{0.4}\) composite (b) and FA (c)](image)

### 3.3. Antibacterial activity

Fusidic acid (FA), an antibiotic produced from the *Fusidium coccineum* fungus, belongs to the class of steroids, but has no corticosteroid effects [29]. FA was characterized as an \(\alpha, \beta\)-unsaturated carboxylic acid; its molecular formula is \(\text{C}_{31}\text{H}_{48}\text{O}_{6}\), containing one acetoxyl and two hydroxyl groups (figure 4).
FA has been widely used in the systemic and topical treatment of staphylococcal infections, including strains resistant to penicillin and other antimicrobials, making it an alternative for the treatment of diseases caused by MRSA strains [29, 30]. Furthermore, it is a hypoallergenic agent, has low toxicity, shows low resistance, and has no cross-resistance with other clinically used antibiotics [29].

In this work, the antibacterial activity of FA-loaded BC composites were investigated by disc diffusion method. The prepared composites were placed on a lawn of tested bacteria *S. aureus*. The efficiency of antibacterial activity of BNC/FA composites was estimated by size of appeared clear zones of inhibition around the samples after 1-24 h of exposure (figure 4).

**Figure 4.** Chemical structure of fusidic acid.

**Figure 4.** Antimicrobial activity of BNC/FA composites against *S. aureus* after 24 h of exposure: BNC 0.1 (a); BNC 0.2 (b); BNC 0.3 (c); BNC 0.4(d); after 1 h of exposure BNC 0.4 (e); after 2 h of exposure BNC 0.4 (f); control FA 0.4 (g).
While within 24 h exposure, diameters of inhibition zones were 27 mm, 30 mm, 32 mm and 34 mm for BNC<sub>0.1</sub> (a); BNC<sub>0.2</sub> (b); BNC<sub>0.3</sub> (c); BNC<sub>0.4</sub> respectively. BNC<sub>0.4</sub> has the best antibacterial activities. This composite inhibited <i>S. aureus</i> growth at the area with diameters 30 mm and 32 mm even after 1 (e) and 2 h (f) exposure of antibiotic with BC, respectively. The obtained results indicate that BNC/FA composite membranes have excellent antibacterial activities against <i>S. aureus</i>.

4. Conclusions
In summary, FA-loaded BNC biocomposites with antibacterial activity were prepared and investigated. FTIR spectrum of the BNC/FA composites indicates presence of the FA in the obtained composites. BNC/FA biocomposites have high antibiotic activity against <i>Staphylococcus aureus</i> and can be used in medicine as a wound dressing.

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