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Bacterial Cellulose/Alginate Nanocomposite for Antimicrobial Wound Dressing

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Abstract

Development of novel wound dressing has attracted more and more attentions in recent years. Bacterial cellulose is a biopolymer of great potentials, which features a distinctive three-dimensional structure consisting of an ultrafine network of cellulose nanofibers. In the present study, nanocomposite bacterial cellulose films modified in situ by the addition of alginate during the static cultivation of *Gluconacetobacter sucrofermentans* B-11267 were produced and then enriching the polymer with an antimicrobial agent tetracycline hydrochloride. The structure of bacterial cellulose and nanocomposite was analyzed by AFM and FTIR. The FTIR spectra displayed the specified interaction between the hydroxyl group of cellulose and the carboxyl group of alginate. The produced bacterial cellulose and nanocomposite were analyzed to determine tensile modulus. The antibacterial activity of nanocomposites were investigated by disk diffusion method. The resulting nanocomposite have high antibiotic activity against *Staphylococcus aureus* and can be used in medicine as a wound dressing.

Keywords: bacterial cellulose, *Gluconacetobacter sucrofermentans*, alginate, nanocomposite, antibacterial activity, wound dressing

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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 [1]. Bacterial cellulose (BC) has a unique nanosized 3D network from thin fibers with diameter more than 100 times smaller than that of plant-derived fibers [2-4]. This unique micromorphology enables it to have large surface area, great

water holding capacity, high porosity, excellent mechanical strength (Young's modulus of single fibers: 2 GPa), high moisture content (>90%), crystallinity (70–90%), thermal stability (up to 300°C) and degree of polymerization (up to 10,000 anhydroglucose units), which determines its potential application in the biomedical and pharmaceutical fields [2-6]. BC has been widely applied in the medical field because of its biocompatibility and has been fabricated as a potential scaffold for cartilage tissue engineering, artificial blood vessels for microsurgery and artificial skin for wound healing [7-11]. Bacterial nanocellulose (BNC) as controlled drug-delivery system has drawn tremendous attention over the last 10 years [6].

Alginate (Alg), another natural polysaccharide product, is extensively used in many applications such as scaffolds and wound dressings due to its biocompatibility, biodegradability under normal physiological conditions and capacity for bioresorption of the constituent materials [11]. Alg hydrogel matrix can be easily fabricated by electrostatic crosslinking with divalent ions such as calcium and barium ions. Recently, hybrid nanocomposites of BC and alginate were developed for use as scaffolds for tissue engineering, films for wound dressing and supports for drug delivery [11-13].

However, both bacterial cellulose and alginate are lack of antibacterial property which limits the possibilities of application in wound dressing areas. There are several successful attempts to impart antimicrobial properties to BC. Bacterial cellulose-silver nanocomposites were successfully prepared and they exhibited excellent antibacterial activity [13, 14]. The synthesis of BNC/chitosan composites with high mechanical reliability and antibacterial activity was reported [15]. Antibiotics are used in combination with BC for the preparation of composite membranes with antibacterial activity [16, 17].

The aim of this study was to prepare in situ bacterial cellulose/alginate nanocomposites and evaluate their structure, mechanical strength and antibacterial activity.

2. Materials and methods

2.1. BC Preparation

BC 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 [18]. For BC 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 for 20 min at 120 °C. 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. BC was produced in static conditions at 28 °C for 5 days. After incubation, BC was collected, washed thoroughly with de-ionized water to remove medium components, and treated with 1 % (w/v) sodium hydroxide solution for 1 h at 80 °C to eliminate bacterial cells. Further, BC was rinsed extensively with 6 % (v/v) acetic acid and then with de-ionized water until pH became neutral. The purified BC was dried to constant weight at 60 °C.

2.2. Production of BC/Alg nanocomposite

Nanocomposite bacterial cellulose films modified in situ by the addition of 2 % (w/v) alginate during the static cultivation of *G. sucrofermentans* B-11267 for 5 days at 28 °C were produced and cross-linked by an aqueous solution of 5% $CaCl_2$ for 3 h. Further, nanocomposite was rinsed by de-ionized water to remove the excess cross-linking agents. Then nanocomposite films were cut into round shapes with 10 mm diameter and immersed in 10 mL aqueous tetracycline hydrochloride (TCH) solution with concentrations of 0.1, 0.2 and 0.3 g/L for 24 h. Then they were dried at 60 °C for 24 h. The final BC/Alg/TCH nanocomposite films were named as BC/Alg/TCH $_{0.1}$, BC/Alg/TCH $_{0.2}$ and BC/Alg/TCH $_{0.3}$, respectively.

2.3. Characterization

The surface morphology of BC and BC/Alg nanocomposite was studied by contact atomic force microscopy (AFM) using an SPM 9600 (Shimadzu, Japan). FTIR spectra of BC, Alg and BC/Alg nanocomposite were obtained using a Fourier transform infrared spectrometer IRPrestige-21 (Shimadzu, Japan) in absorption mode. The tensile strength of the BC and nanocomposite was measured by a Universal Testing Machine- XLWPC (Blacmer, China – USA). The sample was cut into strip-shaped specimens with a width of 20 mm and a length of 50 mm. The stretch rate was 2 mm/min.

2.4. Antibacterial activity

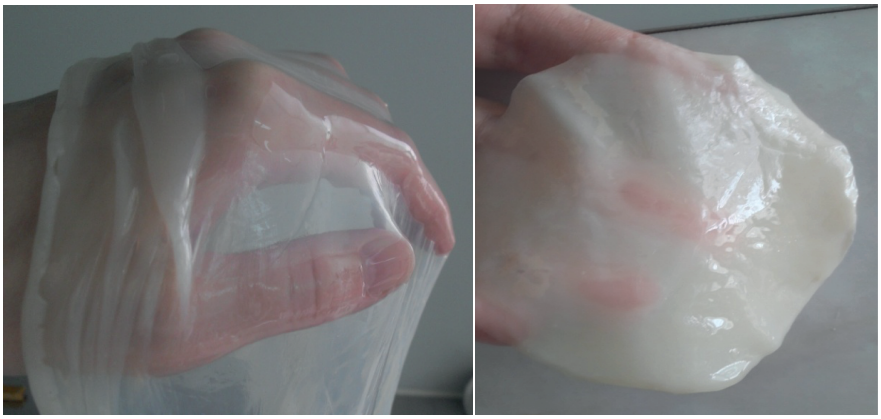
The antibacterial activity of BC/Alg/TCH nanocomposites was investigated by disk diffusion method against *Staphylococcus aureus* 209 P. Lawns of test bacteria (about 1×10^5 CFU/plate) were prepared on medium N1 GRM for bacteria cultivation. The sterilized samples were then carefully placed upon the lawns. 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 BC and BC/Alg nanocomposite

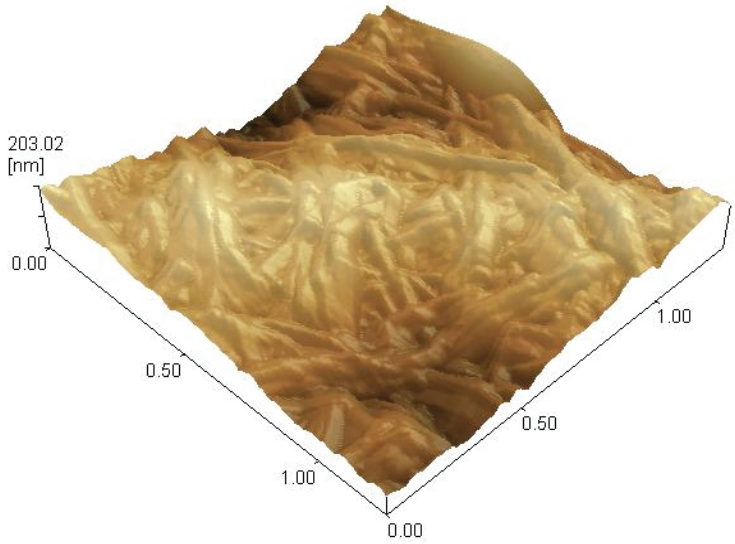
The macrostructure morphology of BC varied depending on the different culture methods. In stationary culture, a gelatinous cellulose film is formed on the air/liquid interface of the medium. In agitated culture, cellulose is synthesized in deep medium in the form of fibrous suspensions, pellets, irregular masses [19]. When cultivating bacterium *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 (Fig.1 A). Nanocomposite bacterial cellulose films modified in situ by the addition of 2 % (w/v) alginate during the static cultivation of *G. sucrofermentans* B-11267 were produced and cross-linked by an aqueous solution of 5% $CaCl_2$(Fig.1 B).



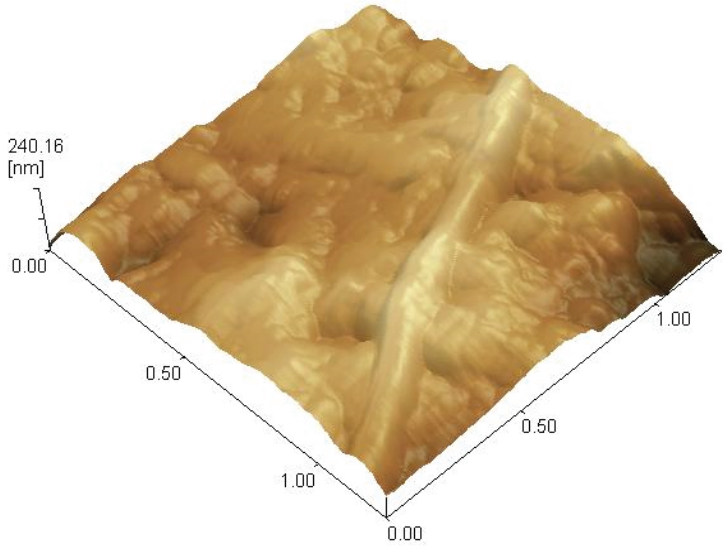
A B

Figure 1: BC gel film (A) and BC/Alg nanocomposite (B).

To study the microscopic details of the biopolymer, atomic force microscopy (AFM) was used. The BC film surface showed a typical bacterial cellulose network composed of long and entangled cellulose nanofibrils (Fig. 2 A) [8, 12]. As previously reported the thickness of BC fibrils formed by *G. sucrofermentans* B-11267 on standard HS medium averaged 50-90 nm [17]. The BC/Alg nanocomposite had a more homogenous and gelatinous structure with a more closed network (Fig. 2 B). This result was consistent with the work of Cacicedo et al. [12].



A



B

Figure 2: AFM image of the BC (A) and BC/Alg nanocomposite (B).

3.2. Mechanical strength

The information of mechanical properties of the dry and wet materials could be used for a guide for selection or modification for wound dressing applications. The tensile strength and elongation at break of the materials in dry and wet state are summarized in Table 1. For all of the examined materials, dry BC had the highest tensile strength

because of its nanofibril network structure with strong hydrogen bonding [20]. The tensile strength of air dried BC was reported in the range of 129-198 MPa [21], whereas those of freeze dried BC was in the range of 8-14 MPa [22]. In this study the mechanical strength of BC/Alg nanocomposite in dry state (27.41 MPa) was lower than that of BC (97.95 MPa) but had relatively higher elongation at break (14.7 %) which indicates flexibility. It has been previously suggested that the intermolecular interaction between the hydroxyl group of BC and the carboxyl group of Alg might reduce the crystallinity and mechanical strength of the composite BC-Alg materials [11, 12]. They are composed of layers of Alg and BC-Alg respectively, in which Alg chains were crosslinked by Ca^{2+} through ionic interaction between the cations and the carboxyl groups. The tensile strength of BC/Alg nanocomposite in wet state (2.05 MPa) was relatively higher than that of BC (0.66 MPa) but had lower elongation at break (26.55 %).

Table 1: Mechanical properties of the wet and dry materials.

Materials		Thickness (mm)	Tensile strength (MPa)	Elongation (%)
Wet	BC/Alg nanocomposite	0.323±0.014	2.05±0.1	26.55±0.4
	BC	0.561±0.085	0.66±0.1	63.7±1.8
Dry	BC/Alg nanocomposite	0.065±0.002	27.41±0.3	14.7±0.8
	BC	0.020±0.001	97.95±1.6	4.10±0.5

3.3. FTIR spectroscopy

FTIR spectroscopy was used to investigate the potential interactions between Alg and BC in the BC/Alg nanocomposite (Fig. 3). The FTIR spectra of the Alg, BC/Alg nanocomposite and BC were measured at wavelengths of 4000–650 cm^{-1}. Pure alginate showed characteristic peaks centered at 1609 and 1400 cm^{-1}, which are commonly assumed to be asymmetric and symmetric carboxyl stretching bands, respectively. The carboxyl group band for the BC/Alg nanocomposite was shifted from 1609 to 1604 cm^{-1} and from 1400 to 1396 cm^{-1}. The result, therefore, implied that there might be some specified interaction between the hydroxyl group of cellulose and carboxyl group of alginate.

The observed band shifts in the BC-Alg spectrum could be attributed to the intermolecular hydrogen bonds based on the functional groups of alginate and cellulose, rich in carboxylate and hydroxyl groups, respectively [12]. These results suggest that alginate plays a crucial role in the biophysical properties of the BC matrix. The

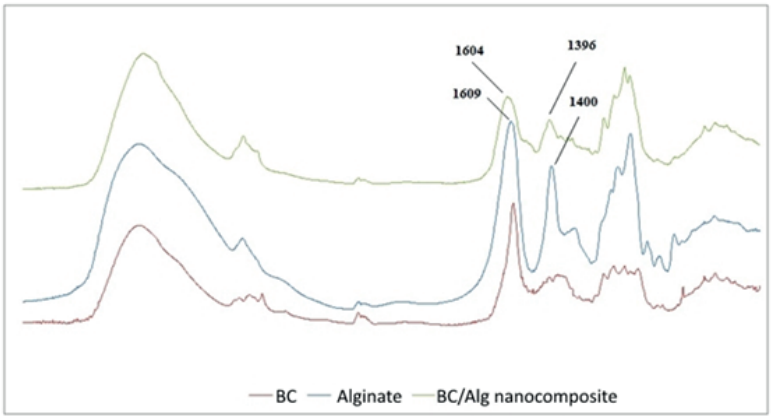


Figure 3: FTIR spectra of BC; Alg and BC/Alg nanocomposite.

presence of alginate in the BC network exposes more hydrophilic groups that could further increase the film hydrophilicity [12]. The increase of hydrophilicity in the BC film is advantageous since it can be related to the drug loading rise of water-soluble molecules such as tetracycline hydrochloride (TCH).

3.4. Antibacterial activity

Tetracycline hydrochloride includes a group of broad spectrum antibiotics. In this work, the antibacterial activity of TCH-loaded BC/Alg nanocomposites 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 nanocomposites was estimated by size of appeared clear zones of inhibition around the samples after 24 h of exposure (Fig. 4). While within 24 h exposure, diameters of inhibition zones were 21±1 mm, 24±1 mm and 26±1 mm for BC/Alg/$TCH_{0.1}$ (A); BC/Alg/$TCH_{0.2}$ (B); BC/Alg/$TCH_{0.3}$ (C), respectively. The obtained results indicate that BC/Alg/TCH nanocomposites have excellent antibacterial activities against *S. aureus.*

4. Conclusions

In summary, TCH -loaded BC/Alg nanoocomposites with antibacterial activity were prepared and investigated. FTIR spectrum of the BC/Alg nanoocomposites indicates interactions between Alg and BC in the nanocomposite. BC/Alg/TCH nanocomposites have high antibiotic activity against *Staphylococcus aureus* and can be used in medicine as a wound dressing.

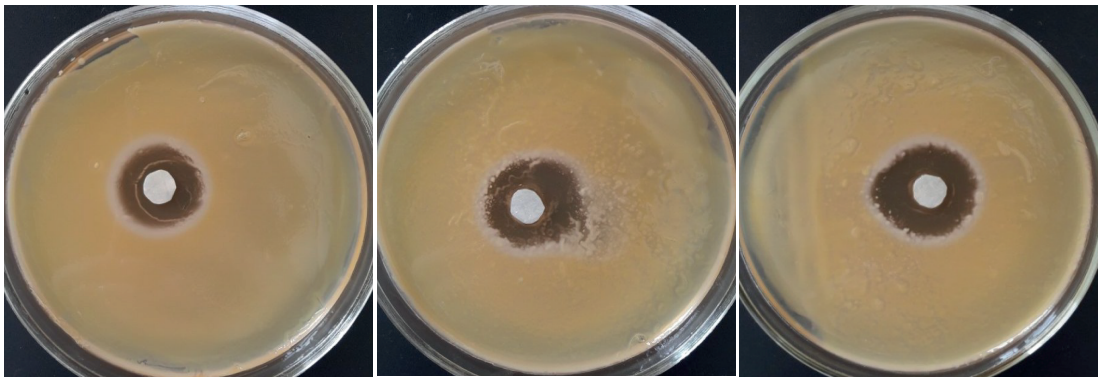


Figure 4: Antimicrobial activity of BC/Alg/TCH nanocomposites against S. aureus: BC/Alg/$TCH_{0.1}$ (A); BC/Alg/$TCH_{0.2}$ (B); BC/Alg/$TCH_{0.3}$ (C).

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