Physicochemical and Antibacterial Properties of Composite Films Based on Bacterial Cellulose and Chitosan for Wound Dressing Materials

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Article info	Abstract
	New bacterial cellulose/chitosan (BC/Ch) nanocomposite films were obtained using a simple procedure by immersing BC synthesized by *Komagataeibacter xylinus* in 1% acetic acid solutions of Ch with the degree of deacetylation 75–85% of medium molecular weight. The BC and BC/Ch composites chemical composition was examined by FTIR, the mechanical properties by a tensile tester, surface morphology by scanning electron microscopy, and antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* by diffusion and joint incubation methods. The FTIR spectra indicated the intermolecular interaction between BC and Ch. Due to addition of 0.6% (w/v) Ch, the films of BC/Ch become more homogeneous with a significantly denser fibril structure, smaller pore diameter and higher surface area in comparison to those of pure BC films. Micro- (15–35 nm) and macrofibrils (50–150 nm) in both BC and BC/Ch films are joined in ribbon-like fibers, providing a high degree of mechanical strength (Young's modulus: 33–36 MPa, tensile strength and elongation et break: 17, 22 MPa). The obtained hybrid material is transparent, flexible and displays good water absorption capacity and water vapor permeability. The films have reasonable thermal stability to be in contact with body or during steam sterilization, since maximum degradation temperature (Td) of both biocomposites is around 400–600 °C. The disc diffusion method confirmed that the BC/Ch films have predominantly non-diffusible antibacterial properties. Antibacterial assessment by the joint incubation method proved that addition of Ch to BC films resulted in significant growth inhibition against target bacteria. The BC/Ch biocomposites' notable properties make them suitable for wound healing applications.

1. Introduction

One of intensively developing directions of materials science is creation of biocomposites. In this area the popularity is raised by the researches directed to development of composite materials on the basis of bacterial cellulose (BC) biosynthesized by *Komagataeibacter xylinus* [1, 2]. It is used as a medical material as it is biocompatible, non-toxic and has no immunological reactivity, and therefore does not cause allergy and immune rejection [3, 4]. BC have been thoroughly investigated for biomedical applications such as bone-repairing scaffolds, drug carriers and others. Most often BC hydrogel film is applied in medicine as skin tissue repair and wound care materials due to its intrinsic nanofibrillated network structure [5]. It supports an optimum balance of humidity stimulating cell adhesion, is permeable for liquids and gases, can be painlessly applied and removed, absorbs decay products of tissues, and serves as almost insuperable physical barrier to infections [6].

Although BC has been shown to be effective as wound dressing, it has no antimicrobial properties to prevent wound infection [7]. Microfibril units occupy insignificant volume of the BC gel-film, therefore allowing introduction of various molecular systems and medical drugs. Upon preparation of antibacterial films cellulose can be combined with several materials to improve antibacterial properties of cellulose. Creation of the antimicrobial wound coverings usually includes addition of antibiotics and antiseptics, different forms of colloid silver and its nanoparticles [8, 9]. However, many causative

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agents of wound infections have multiple resistance to antibiotics. Therefore, almost all manufacturers search for new classes of inexpensive materials of nonchemical effect and free of antibiotics. There is no resistance to antiseptics, however they only possess antimicrobial activity, no wound healing activity. In this regard, the wound dressing consisting of an agent which has both antimicrobial and tissue regeneration properties, would be very promising.

In recent years protective wound coverings from chitosan have been actively studied. Chitosan (N-deacetylated derivate of chitin) is another natural polysaccharide. It exhibits unique physicochemical properties like air and vapor permeability, antimicrobial activity, biocompatibility and excellent film-forming ability. Being chemically decomposed, Ch releases N-acetyl-β-D-glucosamine leading to fibroblast proliferation and ordered collagen deposition, which ultimately results in faster wound healing process. Ch intensifies an adhesion of a burn and wound surface without seams formation, as stimulates growth of skin collagen fibers providing integument elastance. Moreover, applying of Ch on a wound has hemostatic and analgesic effects [10]. These properties of Ch have attracted scientific and industrial interest in fields such as pharmaceutics, biomedicine and biotechnology [11, 12].

However, pure chitosan films are often brittle and the cost of such a polymer is high, which limits its applications. It seems that a good way to improve mechanical and functionalities properties of Ch films is to create a blend with another natural polymer, such as BC. Such a hybrid material would combine the advantageous properties of both cellulose and chitosan. The addition of Ch on BC primarily aims at increase of mechanical and antibacterial properties of the cellulose.

The objectives of this study were (a) to produce biocomposite films from bacterial cellulose and chitosan possessing an antibacterial effect and (b) to evaluate their physicochemical, morphological and antibacterial properties relevant for their medical application.

2. Experimental

2.1. Microbial strains

The new bacterial strain-producer of BC *Komagataeibacter xylinus* C-3 was isolated from Kombucha. The strain was identified and genotyped by National center for biotechnology (Astana,

Kazakhstan). The strain's Gen Bank accession number is KU598766. All the bacterial strains *Staphylococcus aureus* 209 and S60, *Pseudomonas aeruginosa* 853, *Escherichia coli* 157 were obtained in Biotechnology Department at Al-Farabi Kazakh National University.

2.2. Production and preparation of BC films

Our previous studies showed that the optimal nutrition medium for BC gel film formation by *Komagataeibacter xylinus* C-3 strain under static culture conditions is the HS medium containing 1% glucose, 0.5% ammonium sulfate $(NH_4)_2SO_4$, 1.0% acetic acid, 0.5% ethanol, and 0.1% beer wort. The medium was sterilized at 121 °C for 15 min. Precultures were prepared by transferring 50 mL of a stock culture to 1000 mL of medium in 1500 mL bottles and incubated statically at 30 °C for 5 days. The developed gel-like cellulose pellicle was first purified by washing with deionized water for 5–7 min. Then it was treated with 1% (w/v) NaOH at 35 °C for 24 h to remove bacterial cells and the obtained acellular matrix was rinsed with water until the pH rinsing solution was 6.8–7.2. Afterwards, the purified sheets were air-dried at room temperature and stored in a tightly closed plastic box before use.

2.3. Preparation of BC/Ch composite by the "submerging" ex situ method

Chitosan of medium molecular weight (100–300 kDa) and a degree of deacetylation of 75–85% was purchased from Sigma-Aldrich Chemie GmbH. Chitosan powder was dissolved in an aqueous solution of 1% acetic acid to a final concentration of 0.6%. BC films were placed in chitosan solution and incubated for 6 h at room temperature. The excess of chitosan solution was removed by placing BC film between two sheets of filter paper. The films were stored sterile at a relative humidity of 50% and a temperature of 25 °C.

2.4. Preparation of BC/Ch composite by the "solvent casting" in situ method

Seeding culture broth was added to the main culture medium with 1% Ch dissolved in acetic acid. The 75 mL of medium was put in a Petri dish and incubated at 30 °C for 7 days. The developed gel-like cellulose pellicle was first washed with deionized water and then was dipped into 1%

(w/v) NaOH at 35 °C for 24 h to remove bacterial cells. The washing step was repeated until the pH reached 6.8–7.2. Afterwards, the purified sheets were dried at room temperature.

2.5. Characterization of BC and BC/Ch composites

BC and BC/Ch composites were characterized mainly by Fourier transform infrared spectroscopy (FTIR), electron microscopy, thermogravimetric analysis. In addition, their swelling behavior, water retention, water vapor transmission, thickness, mechanical properties and antibacterial activity were examined as described below.

2.5.1. FTIR studies

FTIR spectroscopy was used to analyse the chemical structure of the composite films and to identify possible interactions between their components. The FTIR spectra of the membranes were measured with a IR-Fourier spectrometer FSM-1201 (LLC «Infraspek», Russia). The spectra were recorded as the average of 50 scans at a resolution of 4 cm^{-1} in the range from 4000 to 400 cm^{-1}. The dried freshly prepared films were clamped in the spectrophotometer perpendicular to the direction of the infrared beam. The results were recorded and represented in the form of the signal intensity as a function of a wave number [13].

2.5.2. Scanning electron microscopy (SEM) studies

The surface morphology of lyophilized BC and BC/Ch samples was observed by field emission scanning electron microscope JSM-7800F (Jeol, Japan). Prior to the SEM observation, the films were sputter coated with a platinum-palladium alloy (Pt/Pd 80/20).

2.5.3. Thickness Measurement

The thickness of the films was measured using a digital micrometer screw gauge (precision 0.001 mm, Shantou Wingtai Packing Equipment Co., Ltd., China). The average of ten points from different regions of the films was determined and reported as the mean film thickness with standard deviation.

2.5.4. Mechanical tests

The tensile strength and the Young's modulus were calculated from the stress-strain data by an universal uniaxial tensile tester machine (Instron, USA) [14]. Each sample was cut into dumbbell-shape. Measurements were carried out at temperature (25 ± 2) °C and relative humidity (55 ± 5) %, having sample deformation rate set to 100 mm/min. In each measurement, the average values of at least three independent specimens were taken.

2.5.5. Thermogravimetric analysis (TGA)

TGA of BC and BC/Ch films was performed to evaluate their thermal degradation profiles and thermal stability. TGA was carried out with a NETZSCH STA 409 PC/PG system (using a Shimadzu TGA 50 analyzer). All analyses were performed with a 10 mg sample in alumina pans under a dynamic nitrogen atmosphere between 30 and 1.000 °C. The experiments were run at a scanning rate of 10 °C/min.

2.5.6. Water absorption capacity (WAC)

The water absorption of films was determined by a gravimetric method. The samples were cut into 2 cm × 2 cm pieces, dried to constant weight and their dry weight (Wd) was measured. The dried membranes were immersed in water (or wound exudate) at room temperature. At certain intervals (30–45 min), the swollen specimens were withdrawn from the excess water at the surface of membranes by blotting it out with filter paper. The weights of the swollen films (Ws) were measured, and the procedure was repeated until no further weight change was observed.

2.5.7. Water vapor permeability measurement

The water vapor transmission rate (WVTR) of the dry BC and BC/Ch films with area of 50 cm², were determined with a Lyssy L80-4000 (Systech Illinois, Switzerland) water vapor permeation tester following ISO 15106-1. The determination of WVTR was done under 35 °C and 90% relative humidity. As water solubilized the membrane and permeated through the sample films, nitrogen gas swept and transported the transmitted water vapor molecules to a calibrated infrared sensor.

2.5.8. Antimicrobial activity studies

Antimicrobial activities of BC and BC/Ch films were studied against *E.coli* and *P.aeruginosa* used

as Gram-negative bacteria models and *S.aureus* as a Gram-positive representative. The antimicrobial assessment was carried out by two methods. In the disc diffusion method the composites of bacterial cellulose were cut into discs with a diameter of 1.5 cm, sterilized by autoclaving for 15 min at 120 °C, and were placed on a surface of agar plates previously inoculated by target bacterial suspensions of *E.coli*, *S.aureus* and *P.aeruginosa*, and then incubated for 24 h at 37 °C and inhibition zones were assessed.

In a "joint incubation" method, dried composites of bacterial cellulose were cut into discs of 1.5 cm in diameter, sterilized by autoclaving for 15 min at 120 °C. Samples were divided into two groups. The first group was placed in 1 ml sterile nutrient broth as sterility control. The second group was placed into a culture medium with targets bacteria at a concentration of 10^5 colony forming units per ml (CFU/ml) and then incubated in a shaker at 37 °C for 24 h. After incubation, 50 ml of physiological solution was added to each of the groups, and then all the samples were shaken again for 2 h. 50 µl of bacterial suspension were taken from each tube, inoculated on nutrient agar and incubated at 37 °C for 48 h to count the colony forming units [15, 16]. The same procedure was performed on pure BC.

3. Results and discussion

Unique features of BC (micro- and nanofibrillar structure, high porosity and crystallinity) create a huge potential for development of various composite materials on its basis [17]. Such composites usually consist of two types of certain materials: a matrix and reinforcements. The matrix works as a carcass and supports the reinforcing material while reinforcement improves physicochemical and biological properties of a matrix. A distinctive feature of such clusters (complexes) is a synergy of natural stabilizing matrix properties and various specific properties of reinforcing materials. There are two main approaches for synthesis of composites: *in situ* and *ex situ* [18]. The *in situ* method, also called solving casting, uses an addition of reinforcing element to polymer during its biosynthesis, and then it becomes a part of a polymer structure. During the *ex situ* synthesis, the already finished polymeric matrix is impregnated with the reinforcing material. Both methods were used during this study.

During cultivation of a producer in static conditions, BC was synthesized in a form of pellicle on the surface of a culture medium. Our previous studies showed that the addition of Ch possessing antibacterial properties in culture medium strongly inhibited the *A. xylinus* growth and BC formation (data not shown). It should be noted that this method could be successful either with application of Ch of a molecular weight not more than 30.000–80.000 Da [19], or upon addition of culture medium plasticizers such as glycerol [9], or use of water soluble Ch, obtained by a special chemical treatment [20]. However, the addition of such forms of Ch during BC synthesis reduced the mechanical properties of cellulose and antibacterial properties of chitosan itself [21].

The use of the *ex-situ* technology made it possible to obtain BC/Ch biocomposite gel films (Fig. 1). The mass ratio of BC and chitosan was 75:25. In other words, the weight percentage of chitosan relative to the BC/Chitosan composite was 25%. By immersing the BC of gel in a solution of chitosan, multiple layers of water surrounding the polyglucosan chains will shift, inducing the formation of bonds between cellulose and chitosan, as a consequence, changing the structure of the film [22]. Thus, the chemical structure of chitosan should allow it to easily integrate in the bacterial cellulose structure, presumably forming a structurally strong material.

The resulting film samples were homogeneous and transparent (Fig. 1). The film thickness was 40 ± 2 µm. The films were stored at a relative humidity of 50% and a temperature of 25 °C. It is shown that BC/Ch films have a flat and smooth surface. At sufficient humidification such films could be removed from a wound easily, without injuring a «fresh» epithelium.

Fig. 1. The appearance of the obtained BC/Chitosan films.

The structure, pore morphology, tensile strength, chemical structure and anti-microbial ability of the developed films were then examined to investigate the effect of chitosan supplement.

Since the molecular structure of BC and Ch is very similar, it is expected that these two polymers have good compatibility and miscibility. The FTIR spectra of BC membrane and BC/Ch film are presented in Figs. 2 and 3.

Characteristic absorption peaks of bacterial cellulose are at 3200–3500 cm^{-1} due to O-H stretching. Band at 2987.1 cm^{-1} represents the aliphatic C-H stretching vibration. The band at 1652.2 cm^{-1} is due to deformation vibration of the absorbed water molecules [23]. A sharp and steep band observed at 1.080 cm^{-1} is due to the presence of C-O-C stretching vibrations.
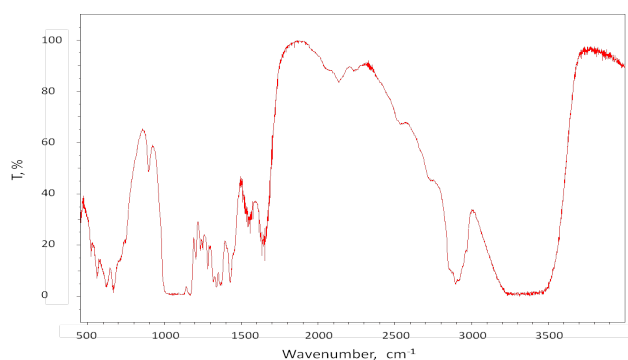
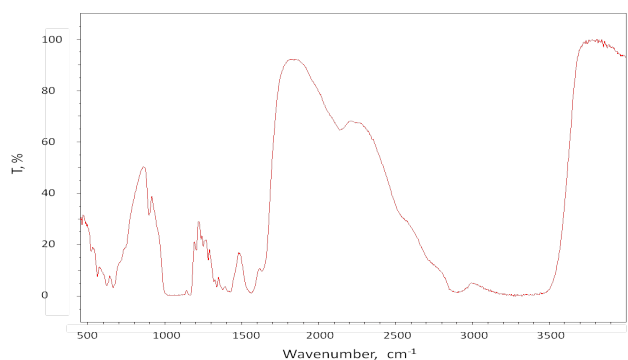Fig. 2. FTIR spectrum of pure BC.

Fig. 3. FTIR spectrum of BC impregnated with chitosan acetic solution.

Figure 3 represents the spectrum of BC membrane impregnated with chitosan solution. Even though the bacterial cellulose membrane is only impregnated with chitosan, some differences are visible in the spectrum of the composite material. In several papers was stated that for composite materials overlapping and/or shifting of absorption peaks are possible [9, 24, 25]. The absorption band at 3200–3500 cm^{-1} shifted to 3034-3491.6 cm^{-1} and became border, indicating a possible overlapping stretching of hydrogen bounded –OH and NH$_2$. Characteristic bands at 2987.1 cm^{-1} for BC typical for CH stretching, shifted to 2899 cm^{-1}. In the cellulose containing chitosan, vibrations of amino groups at 1560.3 cm^{-1} characteristic of chitosan are visible. The peaks at 1.650 cm^{-1} and 1560.3 cm^{-1}, which correspond to saccharide structure of chitosan, is also present in the composite spectrum. These new bands observed at 1650 cm^{-1} and 1560.3 cm^{-1} are attributed to amide-I, amide-II respectively, which exist in Ch molecules.

All two spectra are still visible in the area around wave number 1570 cm^{-1} indicating the presence of aromatic rings. Bacterial cellulose has functional group –OH, -CH, -CH$_3$ bending vibration, and β-1,4-glucan. The addition of chitosan can cause the appearance of C = O stretching (amide I) and-NH bending (amide II) that are characteristic of group C = O amide I and amide II NH group. This indicates that chitosan has interacted with bacterial cellulose, but amide groups of chitin are not deacetylated perfectly. Also, the band at 2940 cm^{-1} is common peak for two polymers attributed to –CH stretching.

FTIR investigation of the obtained films reveals that there is possible chemical interaction between the two biopolymers. Presence of OH group in a cellulose chain leads to bonding with the condensing material (Fig. 4). It is assumed that the chemical modification of bacterial cellulose consists in introducing the glucosamine and N-acetylglucosamine units into the cellulose chain [26].
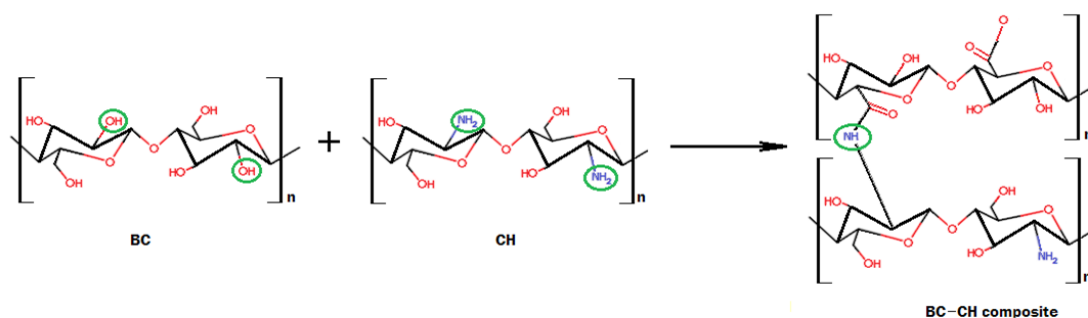

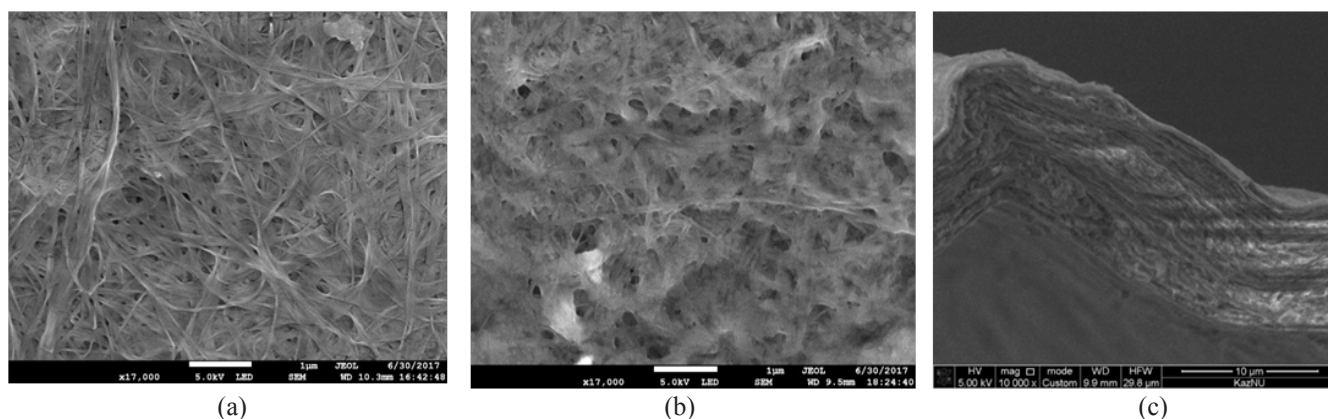Fig. 4. Schematic illustration of the chemical structure BC/Ch composite.

Fig. 5. SEM images of BC (a): surface morphology and BC/Chitosan (b): surface morphology, (c): cross-section morphology.

Figure 5 presents typical SEM images of BC and BC/Ch composite.

As well as with other researchers, the characteristic tridimensional fibrillar network of BC was clearly observed on the film surfaces [1, 3, 19, 27].

This is particularly evident in a cross section picture. The SEM micrographs also provided evidence of good dispersion of BC fibrils on the matrices, without noticeable aggregates. The surface of BC films displayed a typical granular morphology. The BC sample has porous morphology, which has been subject of discussion in the last decade and already established [20, 28].

The surface morphology of BC was changed after Ch treatment. A thick layer of Ch covered the film, so it was difficult to observe individual BC nanofibers. Diameter of the microfibers was about 41+/- 4 nm. BC and BC/Ch microfibrils are further joining together into one millionth centimeter thick ribbon-like fibers. Besides, the presence of uniformly distributed fibers of the carcass is evident, which ensures high strength of the films. On cross section picture, interaction of Ch and BC molecules is noticeable. A high amount of hydrogen bonds formed by the hydroxyl groups caused the pure BC nanofibres to entangle or aggregate, thereby leaving crevices in the microstructure. The porous structure of the film is remained: it has macropores and mesopores of 20–1000 μm in diameter as well as nanopores (up to 4 nm). A similar picture was observed by other researchers [29, 30]. Presence of different pore sizes in a gel-film provides vapor and gas permeability, and also greatly contributes to the growth of epithelium and vascularization. This justifies using BC/chitosan composite as a scaffold for tissue engineering.

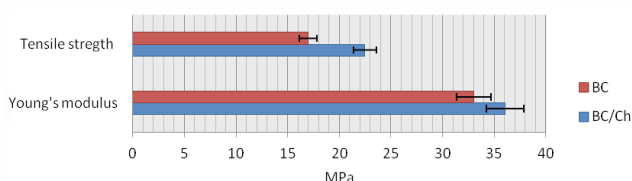The important factor for *ex-situ* method application for composite synthesis is the invariable fi-

Fig. 6. Mechanical properties of bacterial cellulose and BC/Chitosan gel-films; n = 8. The error bars show standard deviation.

brous structure of BC. Moreover, Ch strengthens BC filaments, increasing their mechanical strength. The tensile strength (MPa) and Young's modulus of the obtained composites are presented in Fig. 6.

The robust constructional BC material of high crystallinity degree should be more resistant against high pressure, than a material having irregular structure. Since Ch has an amorphous crystalline state, it was supposed that this property would affect mechanical characteristics of a BC/Ch film. It is shown in Fig. 6, the tensile strength for BC is 17.01 ± 0.50 MPa and for BC/Ch film is 22.48 ± 0.20 MPa.

Therefore, the BC/chitosan biocomposite possessed higher mechanical strength in comparison with BC. The Young's modulus (measure of the stiffness) of BC/Ch and BC is 36.03 ± 1.80 and 33.03 ± 1.65 MPa, respectively, and although the confidence intervals partially overlap, nevertheless t-test showed that a statistically significant difference between these values is at a probability level, $p < 0.5$. These indexes are higher than the values of other flat orientated organic polymer layers [31].

Thus, chitosan had a favorable impact on the modified bacterial cellulose mechanical properties. High elongation at break provides good elasticity of the bacterial cellulose, which is very important for the medicine. An elastic dressing fitting the
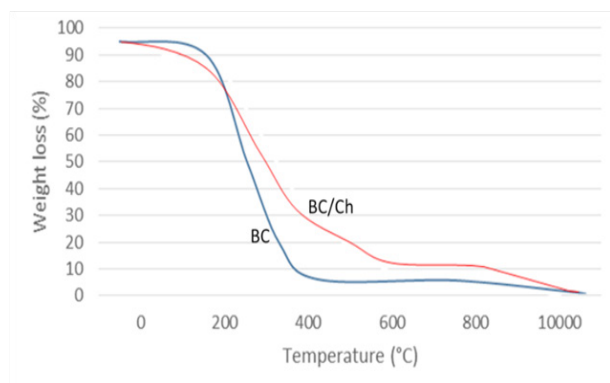
Fig. 7. The TGA spectra of BC and BC/Ch composite.

Thermogravimetric analysis (TGA) of BC and BC/Ch films was performed to evaluate their thermal degradation profiles and thermal stability. Thermo-gravimetric analysis (TGA) is a continuous process, involving the measurement of sample weight in course of temperature in the form of programmed heating. Since TGA provides better understanding of thermal decomposition behavior, the thermal stability and thermal decomposition of the BC and BC/Ch composite investigated using TGA are given in Fig. 7.

Based on Fig. 7, BC/chitosan bicomposite has higher stability compared to the pure BC. In the thermograms of BC and BC/Ch the two mass losses, at around 100 °C and 220 °C, were associated with the volatilization of water and acetic acid. The maximum degradation step at 400–600 °C was assigned to the degradation of polymers. The pure BC started to decompose at around 220 °C. The percentage weight loss for BC at 250 °C was 31.21%, while for BC/Ch composite it was 25.57%. In the case of BC, 50% weight loss was noticed at 270 °C, while the BC/Ch composite was 300 °C.
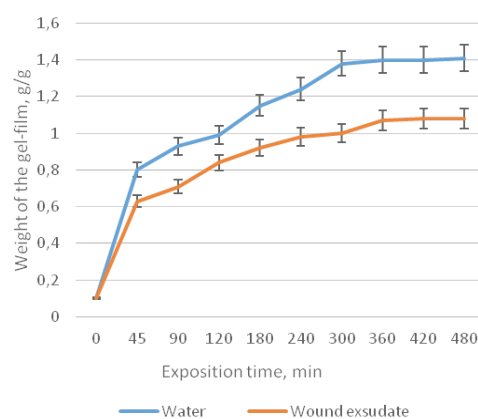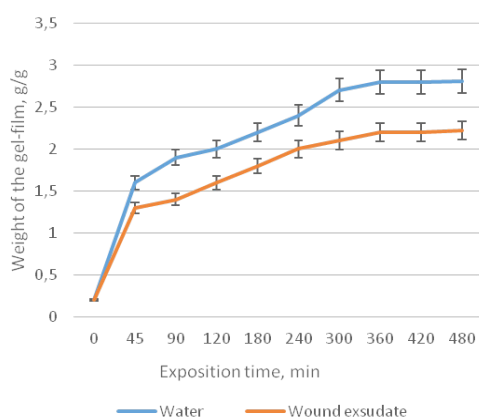
Major weight loss of chitosan is seen between 200 and 350 °C. 50% weight loss can be seen at 330 °C. The weight loss temperature of BC/Ch is higher than that of BC but lower than the Ch. In general, the addition of Ch to BC matrices resulted in a slight increase in the thermal stability of the films.

One of the primal problems requiring the solution at early wounding stages is the sorption of the wound contents, including products of microbial and tissue decay [32]. Therefore, an important criterion in the choice of wound dressing materials is a sorption capacity, which is defined by the ability to absorb maximal quantity of toxins, bacteria, wound contents and other substances.

Microfiber ribbons allow to keep a huge amount of water in the matrix of the BC nano-gel film – from 100 to 200 g of water on 1 g of dry polymer, keeping at the same time a high characteristic tensile strength (17 MPa) [20].

The absorption properties of BC and BC/Ch biocomposite material were defined by weighing the films preliminarily wrung out from liquid, before and after placing them in absorbate. For absorbate saturation time establishment and sorption ability estimation of wrung out gel films kinetics of a distilled water sorption and wound exudate were determined. Results of the experiment are given in the Fig. 8.

The quantity of the absorbate was calculated as a difference of masses of film impregnated with water or an exudate before and after absorption. The experimental data for both liquids showed that process almost finished within 6 h. BC film sorbing the water had the weight of 2.8 ± 0.2 g, BC/Ch – 1.4 ± 0.1 g. Further indexes of adsorption remain at the same level. This means that the maximal amount of pathological liquid exuded from the wound can be absorbed on such films within 6 h, that stipulates terms of dressings.

Fig. 8. Kinetics of water and wound exudate absorption by dehydrated BC (A) BC/Ch (B) films; n = 8. The error bars show standard deviation.

Wound dressings using hydrogel or hydrocolloid sorbents as a basis provide a plasticizing effect on tissues, soften necrotic formations due to tissue rehydration, facilitate mechanical removal of wound detritus, and prevent the development of infection on wound surface and under the scab [33]. Dressings on the basis of such sorbents create a moist environment in the wound, optimal for normal flow of regeneration processes. The water vapor transmission rates (WVTR) of BC and BC/Ch were 1589 ± 1 and 1560 ± 2 g/m^2 day, respectively. Thus, the BC/Ch film had a slightly lower water vapor transmission rate in comparison to the BC film.

Microbial infection can greatly affect wound healing. Antibacterial properties are needed to maximize the usefulness of cellulose as a wound covering material because it can reduce bacterial contamination on cellulose and wounds. Recently, because of a broad unsystematic use of antibiotics, resistance of causative agents of purulent infections significantly increased. The problem may be solved by the development of wound coverings on the basis of biopolmers with antibacterial activity, such as chitosan.

There are two known mechanisms of antimicrobial action of chitosan, both related to the number of active amino groups. First, the positively charged chitosan interacts with negatively charged bacteria, leading to an increase in permeability of bacterial membrane, which leads to inhibition of test strain cells growth. Another mechanism involves the binding of chitosan to DNA, which can suppress the production of bacterial mRNA [34]. Since Ch is antibacterial, the incorporation of Ch in BC should lead to appearance of antimicrobial properties in the BC/Ch composite film. The sensitivity of microorganisms to the modified BC was studied on reference strains of *S. aureus* 209 and S60, *P. aeruginosa* 853, *E. coli* 157, and also associations of *S. aureus* + *P. aeruginosa*. The antagonistic activity of BC/Ch gel films was determined by agar diffusion method and by incubating the film in growing culture of target microorganism. The first method is based on the detection of growth inhibition zones of microorganisms around the disc with the test material.

The pieces of the BC/Ch film were placed on the surface of the cups seeded with a suspension of test microorganisms. After application of the composite, the plates were incubated at 37 ° C for 24 h. The clear growth inhibition zones of the target microorganisms were then measured (Fig. 9).

Inhibitory effect of BC and BC/Ch films was evaluated on the change in a total count of target microorganisms in a liquid after their joint incubation with studied samples (Table).

Table
Antimicrobial activity of BC and BC/Ch films against target microorganisms by joint incubation method

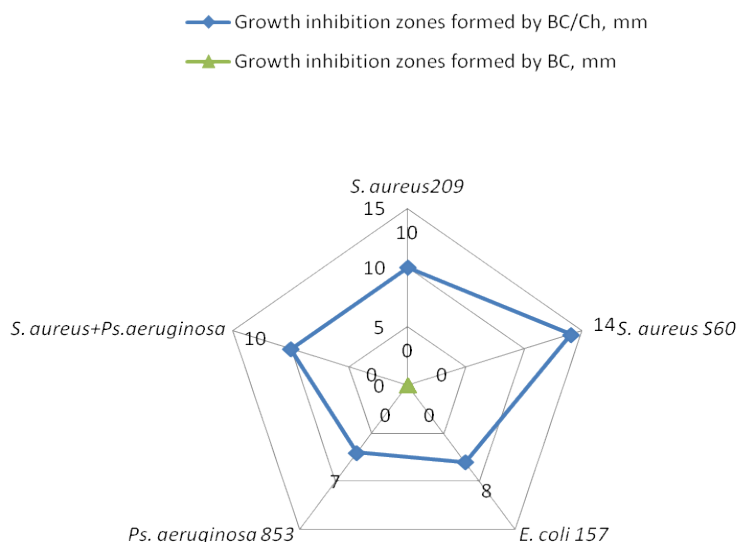| Target microorganisms	Inhibition effect of the films (%)	
	BC/Ch	BC
S.aureus 209	85.1 ± 4.2	2.4 ± 0.1
S.aureus S60	99.3 ± 7.9	4.4 ± 0.2
P.aeruginosa 853	93.2 ± 4.6	1.8 ± 0.1
E.coli 157	98.1 ± 4.9	2.8 ± 0.1
S.aureus+P.aeruginosa	87.1 ± 4.4	1.1 ± 0.1

Fig. 9. Antimicrobial activity of BC and BC/Ch films against target microorganisms by agar diffusion method.

Pure BC itself showed no antibacterial effect in agar diffusion test (p > 0.5). Decrease in microbial count during joint incubation of target bacteria with BC could happen due to their adsorption on a porous matrix of the polymer. Antibacterial assessment in the disc diffusion method indicated that BC/Ch films show only slight antibacterial properties. Inhibition zones were not more than 7–14 mm. On the other hand, BC/Ch composite demonstrated remarkable bacterial inhibition against all used targets evaluated by direct contact with them. These results revealed that the addition of Ch in BC significantly increased the antibacterial efficiency (p < 0.01).

This suggests that Ch tightly incorporated in BC fibrils therefore the antibacterial components did not readily diffuse to the agar to create inhibition zone. Similar data were obtained by other researchers [31, 35]. Since it proves that Ch diffuses onto the plate, the disc diffusion method seems to be not adequate for evaluating its antibacterial activity.

The effective wound dressing should have the following properties. First and foremost it must be able to protect the wound from infection, and secondly it must provide moisturized wound healing environment. With the presence of Ch that is antibacterial, wound closure will be effective to protect the wound and reduce the occurrence of infections. Thus, this research was focused on fabrication of biocomposite film that is the effective as a wound dressing application.

Modification of a bacterial cellulose by immobilizing a chitosan on it results in a composite material with glucosamine and N-acetylglucosamine units incorporated into the cellulose chain, which is characterized by a number of valuable features:
 - good mechanical properties in wet state,
 - high moisture-keeping properties,
 - high antimicrobial activity against Gram (-) and Gram (+) test bacteria.

These features make biocomposite BC/Ch a perspective polymer not only as a wound dressing material but also for tissue engineering.

Acknowledgements

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