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Production and Characterization of Biodegradable Bacterial Cellulose Membranes

Ceyhun BABAC Tülin KUTSAL* Erhan PİŞKİN
Hacettepe University, Chemical Engineering Department and Bioengineering Division, Beytepe, Ankara, TURKEY

*Corresponding Author
e-mail: tkutsal@haccettepe.edu.tr

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Abstract

This study aims to produce and purify biodegradable bacterial cellulose (BC) in a membrane form. A potent cellulose producer, *Acetobacter xylinum* ATCC 10245, was cultivated with glucose statically at 30°C for 15 day. Purification was carried out with a treatment of 0.5 M NaOH. Scanning electron microscopy (SEM) and atomic forced microscopy (AFM) revealed that the fiber diameter was between 50-100 nm. Bacterial cellulose can be converted to dialdehyde cellulose with periodate oxidation for being biodegradable form. Therefore, cellulose films treated with different ratio of periodate/anhydro glucose unit (AGU) (mol/mol), at 50°C, for 24 h in dark media. Degree of periodate oxidation is determined with oxime method related to amount of forming aldehyde group. With increasing of periodate/AGU ratios, degree of oxidation was also increased. Chemical structure of oxidized bacterial cellulose was examined with FTIR spectrum analysis. New band belonged to aldehyde group was observed at 1720 cm^{-1}. Regarding mechanical test results, it was found that the oxidation was caused the decrease in the mechanical properties of BC.

Keywords: Bacterial cellulose, biodegradation, *Acetobacter xylinum*, oxidized bacterial cellulose

INTRODUCTON

Cellulose is one of the most important structural elements in plants as the main component of cell walls. Cellulose from plants is considered impure, containing many kinds of complex carbohydrates. BC presents significant advantages over plant derived cellulose with its structural and mechanical properties. BC has a network structure in which very fine ribbon-shaped fibers composed of a highly crystalline and highly uniaxially oriented cellulose is entangled with one another, and this network structure contains a large quantity of liquid. BC is obtained easier and more pure than plant source cellulose. Plant-derived cellulose and BC have the same chemical composition but different structures and physical properties. Bacterial cellulose differs from plant cellulose with respect to its high crystallinity, high water absorption capacity, and mechanical strength in wet state, ultra fine network structure [1-4].

Up to now several applications of BC in biomedical area are known. BC has been studied for the use as wound dressing and blood vessels [3,5-7]. BC has also a potential to be used as a scaffold for tissue engineering. Recently, BC has been studied for tissue engineering applications with various modifications [8-10].

It is well known that cellulose does not degrade enzimatically or hydrolytically in the human body and no detailed report on its hydrolytic cleavage exists, although cellulose fibers have been cited to be partially biodegradable. The β-1,4 linkage between the glucose units in cellulose is quite stable but could be made susceptible to hydrolysis by introducing chemical modifications. One of this product is 2,3-dialdehyde cellulose (DAC) which is formed by oxidizing cellulose with sodium metaperiodate [9,11,12]. Aldehyde groups created by oxidation are also suitable for many applications, for example attachment of cells, drug, protein and/or peptide or further chemical modification to induce cell adhesion and proliferation [13]. At physiological pH, DAC degrades into glycolic acid and 2,4-dihydroxy butyric acid [11].

In the present study, we have investigated a novel material as a scaffold for tissue engineering. BC ,which is secreted by *Acetobacter xyl inum (=Gluconacetobacter xylinus*) and has unique properties, was used to obtain biodegradable membranes. As a suitable purification method for bacterial cellulose pellicle we used the treatment with diluted sodium hydroxide to eliminate *A. xylinum* cells. The produced BC membranes were treated with periodate to converted in biodegradable form.

MATERIALS AND METHODS

Production of Bacterial Cellulose:

A. xylinum (ATCC 10245) was grown in a media containing 20 g/l d-glucose (Fluka, Germany), 10 g/l peptone (Fluka, Germany), 10 g/l yeast extract Difco (USA), 8mm KH_2PO_4 (Merck, Germany) and 12mm K_2HPO_4 (Merck, Germany). The pH of the medium was adjusted to 5.00. Twenty five milliliters media were inoculated with culture (third cultivation from single colonies) over night at 1 % v/v in 8cm petri dishes and incubated statically at 30°C for 15 days.

Purification of Bacterial Cellulose

The produced BC membranes were washed in 0.5 N NaOH (Merck, Germany) at 80°C for 2 hours to remove bacterial cells and other ingredients, and washed thoroughly with distilled water until neutral pH.

Characterization of Bacterial Cellulose

Approximately 1 mg of dry bacterial cellulose sample was pressed into a pellet with 200 mg of potassium bromide and Fourier transform infrared (FTIR) spectrum was recorded by Schimadzu DR8101 with accumulation of 40 scans at room temperature. The scanning electron

microscope (SEM; Vega Tescan High Vacuum Scanning Electron Microscope, USA) and atomic forced microscopy (AFM; Ambios, Qscope 350, USA) were used for imaging of the nanofibers.

Oxidation of Bacterial Cellulose

The BC membrane, prepared according to the procedure described above, was reacted with sodium metaperiodate (Aldrich, Germany) in water in the dark at 50°C. The mole-to-mole ratio of sodium metaperiodate to anhydroglucose repeat unit (AGU) of cellulose was 0.5, 1.0, 1.5. The membrane was removed after 24 hours and washed first with ethylene glycol and then with distilled water. It was stored in water at room temperature until used. Water was replaced every 12 hours to prevent microbial growth on the membrane.

Characterization of Oxidized Bacterial Cellulose

FTIR analysis was performed as described above with oxidized bacterial cellulose membranes (OBC). OBC membranes were dried at 100°C for 2 h, prior to analysis. Degree of oxidation was determined with oxime method. The amount of reactive aldehydes was subsequently calculated by measuring the oxime formation upon addition of hydroxylamine. The BC and OBC membranes were tested for mechanical properties. Tensile testing was performed on wet state. Specimens were cut with ATSf AAR Vignate-MI-Italy in 10x50 mm dimensions. Changes in tensile strength was determined at room temperature on a Lloyd universal tensile testing machine (Lloyd Instruments, LR 5K Serensworth Fareham, England) operated at cross-head speed of 2 mm/min. Four pieces of each membrane were tested and their mean values were calculated.

RESULTS

Characterization of Bacterial Cellulose

FTIR spectrum of BC was showed in Figure 1. A typical FTIR spectrum of BC was observed, where the absorption band assigned to the hydroxyl group appears 3400cm^{-1} [14]. Other characteristic bands of cellulose were also observed. Functional groups of cellulose and their wave numbers were given in Table 1. There is no clear evidence for aldehyde bands at this stage.

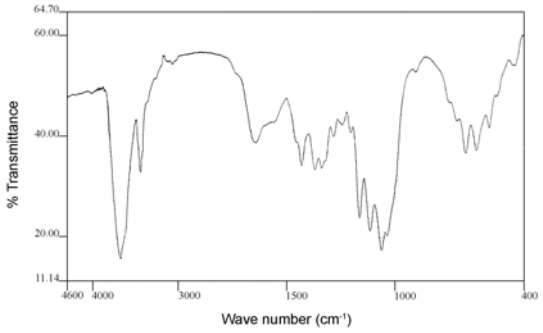


Figure 1. FTIR spectrum of bacterial cellulose

Morphological properties of bacterial cellulose examined with AFM and SEM photography techniques (Figure 2 and 3). Fiber of bacterial cellulose diameter is found to be between 50-100 nm.

Table 1. Functional groups of FTIR spectra of cellulose

Wave number (cm^{-1})	Functional Group
3400	OH stretching, very strong, sharp
2897	CH stretching
1429	HCH and OCH bending inside of plane vibration
1368	CH deformation vibration
899	COC, CCO, and CCH deformation mods stretching vibrations
669	C–OH bending out of plane

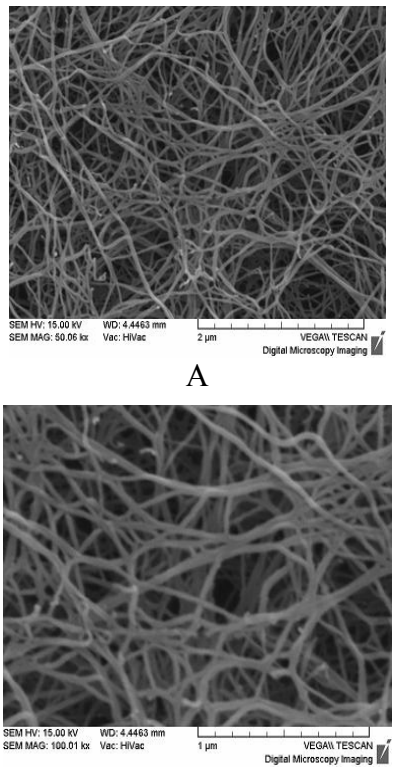
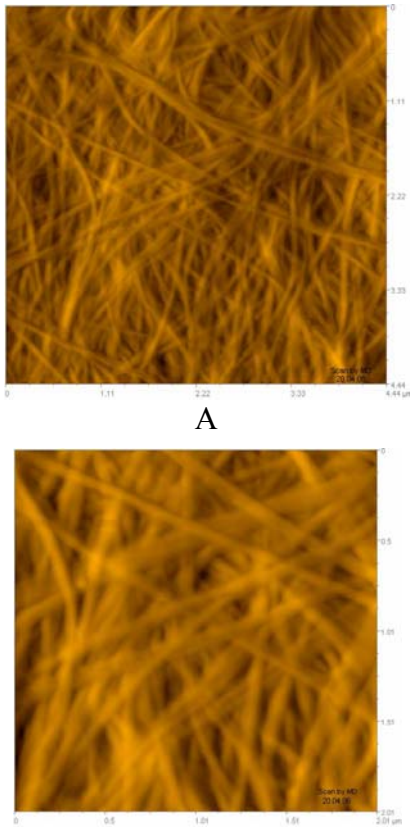


Figure 2. SEM images of bacterial cellulose A) X50000 B) X100000

Characterization of Oxidized Bacterial Cellulose

To prepare the OBC membranes, BC membranes were reacted with periodate in water at 50°C for 24 h. Figure 4 shows the degree of oxidation of OBC membranes made with the use of different ratios of periodate to cellulose at 50°C. The expression degree of oxidation used represents the weight of dialdehyde cellulose millimoles (mmoles) of the aldehyde groups per gram of the membrane. The 100% degree of oxidation means that each anhydroglucose repeat unit in the molecule has been converted into a 2,3-dialdehyde cellulose. Figure 1 shows that the degree of oxidation increases with an increase in the mole-tomole ratio of the anhydroglucose repeat unit to sodium periodate[15]. Degree of oxidation was found 33, 53 and 67 % respectively, for membranes periodate/AGU ratios 0.5, 1.0 and 1.5 (OBC0.5, OBC1.0 and OBC1.5).



A

B

Figure 3. AFM images of bacterial cellulose A) 4.44x4.44 µm B) 2.01x2.01 µm

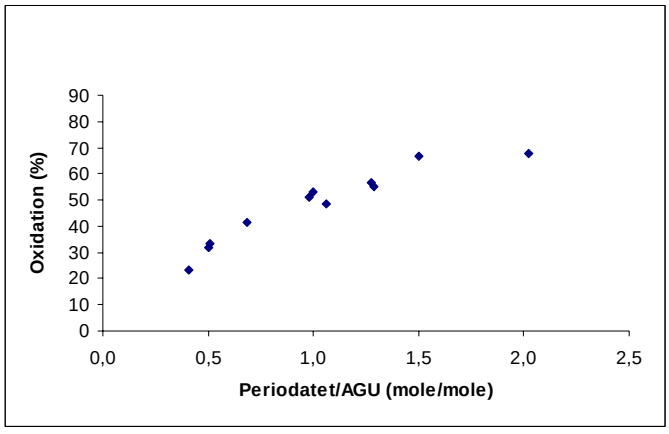


Figure 4. The effect of periodate concentration on degree of oxidation values calculated with the use of the aldehyde content.

Figure 5 compares the FTIR spectra of BC and OBC (degrees of oxidation 27% and 67%) membranes. The three spectra seems to be very similar, except for an additional band at 1720 cm^{-1} due to the carbonyl stretching vibration belonging to the aldehyde group. It must be noted that the detection of the carbonyl stretching vibration band becomes difficult if the membranes are not dry. This is because, in the presence of moisture, the aldehyde group may exist in the hemiacetal, acetal and/or hydrated form(s) [4, 8].

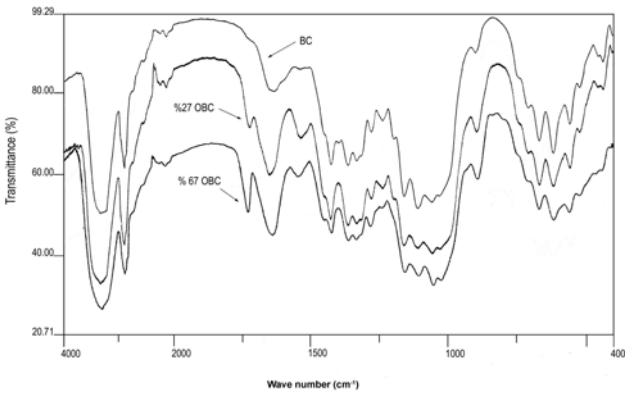


Figure 5. FTIR spectrum of BC and OBC (with the oxidation degree 27% and 67 %)

From the mechanical analysis of the BC and OBC membranes (Table 2), the average tensile strength of BC (157.4) was more than that of OBC. However, the oxidation process used in this study could decrease the mechanical properties of the BC membranes.

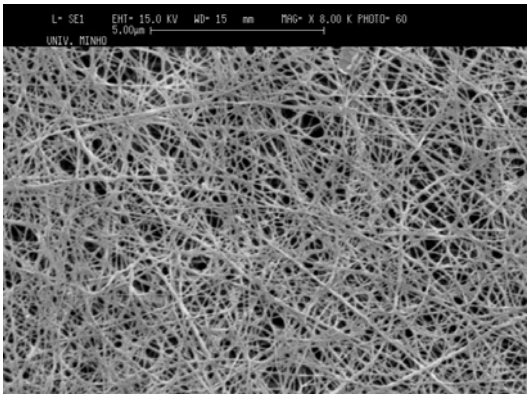
Table 2. Values of tensile strength of BC and OBC membranes

Membrane	Tensile Strength (Pa)
BC	157.4 ± 34.3
OBC0.5	104.3 ± 67.8
OBC1.0	42.2 ± 58.5
OBC1.5	Not detected

As seen on SEM images of BC and OBC (Figure 6), oxidation changed the surface morphology of the membranes. Once, the degree of oxidation increases, size of pores between the nanofibres increase. All membranes showed a reduction in diameter following oxidation. [9].

DISCUSSION

In this study, BC membranes were oxidized with different ratio of sodium metaperiodate in order to obtain biodegradable form. The aldehyde groups present on the OBC membrane serve as sites for cell, drug, protein and/or peptide attachment or further chemical modification to induce cell adhesion and proliferation. The simple methodology for the preparation of these biodegradable OBC membranes can allow them to use in many different biomedical applications.



A

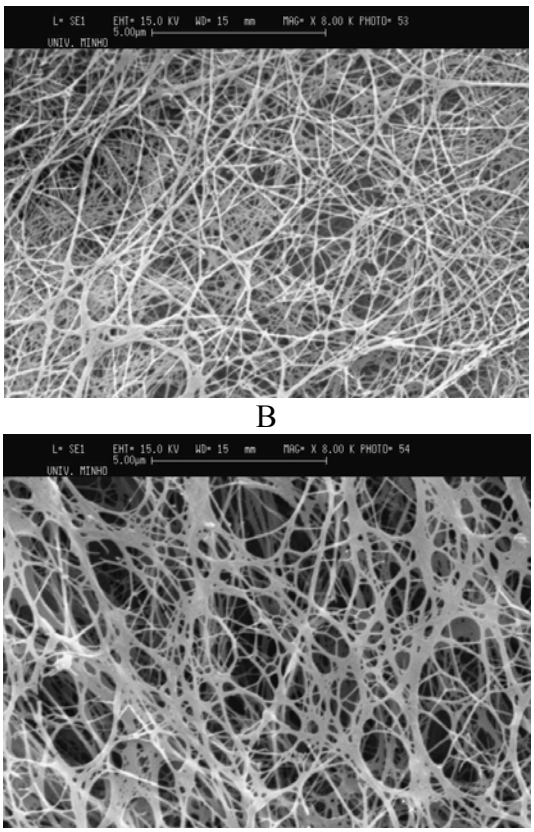


Figure 6. SEM images of A)OBC0.5 (X8000), B)OBC1.0 (X8000) and C)OBC1.5 (X8000) (OBC membranes with Periodat /AGU ratios 0.5, 1.0, 1.5, respectively)

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