



MORPHOLOGY OF DRIED NANOGELE FILMS OF BACTERIAL CELLULOSE IMPREGNATED WITH THE SILVER NITRATE SOLUTION

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A comparative study of dried nanogel films of the *Gluconacetobacter xylinus* cellulose (GXC) in native and disintegrated forms treated with AgNO₃ solution has been carried out by means of SEM and XRD. The supermolecular structure of samples studied was shown to be 3D network of nanostrips formed with oriented macrofibrils in which amorphous and crystalline regions alternate. XRD patterns of the GXC dry films, pre-treated with AgNO₃ aqueous solution, demonstrate both residual AgNO₃ and reduced Ag⁰, the latter positioned in longwise direction of morphologic structure elements. The XRD and SEM studies of dried GXC films disintegrated in 1 % AgNO₃ aqueous solution showed the presence of reduced Ag⁰ in the form of nanoparticles 10 – 50 nm in diameter located in the free volume of the GXC 3D network nearby structural elements.

Keywords: nanogel film; bacterial cellulose; SEM; XRD

Citation: A.K. Khripunov, T.P. Stepanova, N.N. Saprykina, E.P. Astapenko, D.P. Romanov, A.A. Tkachenko, V.M. Kapralova, Morphology of dried nanogel films of bacterial cellulose impregnated with the silver nitrate solution, St. Petersburg State Polytechnical University Journal. Physics and Mathematics. 11(1) (2018) 75 – 81. DOI: 10.18721/JPM.11111

Introduction

The *Gluconacetobacter xylinus* cellulose (GXC) has been heavily studied in the past decade due to its numerous practical applications. Pure sugars and a variety of sugar-containing wastes can serve as potential carbon sources increasing the yield and reducing the costs of this product [1, 2]. However, few publications can be found on the morphology of GXC nanogel films (NGFs) [3] that show great promise, primarily, for their applications in medicine (for example, as wound dressings saturated with various medications), where it is important to gain an understanding of the interaction of silver-based antiseptic with the elements of the supermolecular structure of GXC films [4, 5].

The goal of this work was a comparative study of the morphology of native GXC and its disintegrated form, pretreated with a silver nitrate solution.

Experimental procedure

Sample preparation. Biosynthesis of GXC NGF was described previously in [1, 4]. After cells were removed by the conventional method of boiling in a 1 % sodium hydroxide solution and thorough washing with distilled water, the obtained GXC NGF was stored after sterilization in hermetically sealed containers at a constant temperature of +5 °C until use.

To determine the molecular weight by viscometry, a dry GXC film was dissolved in cadoxen (a cadmium oxide complex in an aqueous solution of ethylenediamine) to obtain molecularly dispersed solutions [6]. The molecular weight of GXC was found to be 3.89·10⁵ Da.

The *Gluconacetobacter xylinus* cellulose film obtained by biosynthesis was dehydrated with a press, removing up to 95% of water. The film was then placed in a 1 % AgNO₃ solution for

about 12 h. The film saturated with this solution was secured in toroidal clamps and dried at room temperature.

The initial native GXC film was disintegrated by adding some distilled water and 100 ml of a 1 % aqueous solution of AgNO_3 (the total volume of the solution was 310 ml). The reaction vessel was a 2-liter blender (JTC, Omniblend 1, model TM-767) with a blade rotation rate of 15,000 rpm. Disintegration was carried out in three stages, each lasting 5 min, with 30-minute breaks to cool the colloidal suspension to room temperature. The gel film of disintegrated GXC was dried in the same clamps as the native one. The resulting dry samples were then studied SEM and X-ray diffraction.

X-ray diffraction. A DRON-3M X-ray diffractometer was used to study the dried initial and disintegrated films. $\text{Cu}_{K\alpha}$ -radiation was used.

Scanning electron microscopy (SEM). The GXC films were examined with a scanning

electron microscope (Supra 55VP, Zeiss, Germany). A 15-20 nm-thick platinum layer was deposited on the samples to maintain the electrically conductive properties of the samples, to eliminate interference caused by accumulation of the surface charge during scanning and to increase contrast. This layer was deposited by cathode sputtering with a turbo-pumped sputter coater (Quorum-150, UK). The samples were then glued to the microscope stage with a double-sided electrically conductive tape. Surface morphology was studied using the secondary electron (SE2) mode.

Energy dispersive X-ray microanalysis (EDXMA). The elemental composition of the samples and the composition of the individual phases were determined using an INCA Energy microanalysis system with an X-Max-80 detector (Oxford Instruments, UK), completed with a Supra 55VP microscope. To identify the phases in the sample, spectra were recorded both from the sample's entire surface and from its individual points.

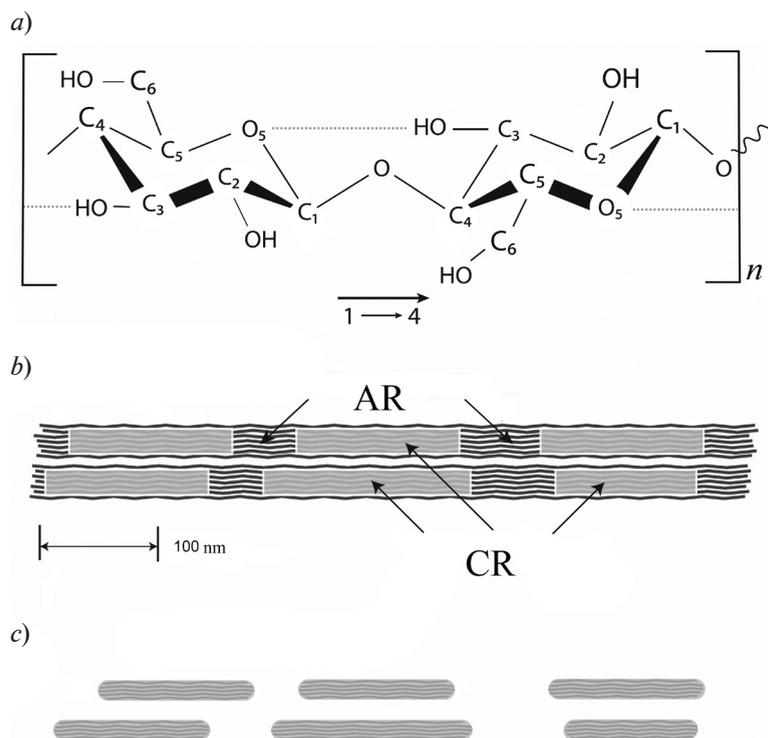


Fig. 1. Schematics of the structure of a monocellulose macromolecule (a), fragment of a macrofibril strip in one of the hypothetical configurations of crystalline (CR) and amorphous (AR) regions (b) and single GXC nanocrystals after dissolution of disordered regions by acid hydrolysis (c). The arrow indicates the direction of the $\text{C}_1 - \text{C}_4$ bond (a)



Experimental results and discussion

Cellulose macromolecules whose monomer unit is cellobiose tend to be mutually ordered due to intra- and intermolecular hydrogen bonds [7 – 11]. The data obtained by XRD analysis, SEM and TEM indicate that the formation of the spatial morphological structure of GXC NGF involves the formation of microfibrils (consisting of about seven orientationally ordered cellulose macromolecules); these microfibrils then become ordered into macrofibrils; the

latter form ordered strips with nanochannels between them.

Due to conformational disorder in the attachment of cellobiose units during biosynthesis, microfibrils consist of alternating crystallites and amorphous regions that microfibril packaging requires to ensure flexibility in the spatial morphological structure of cellulose. Fig. 1 shows the known [8] scheme of the structure of a monomeric unit of a cellulose macromolecule, a strip and single GXC crystallites.

Fig. 2. shows XRD patterns of native and

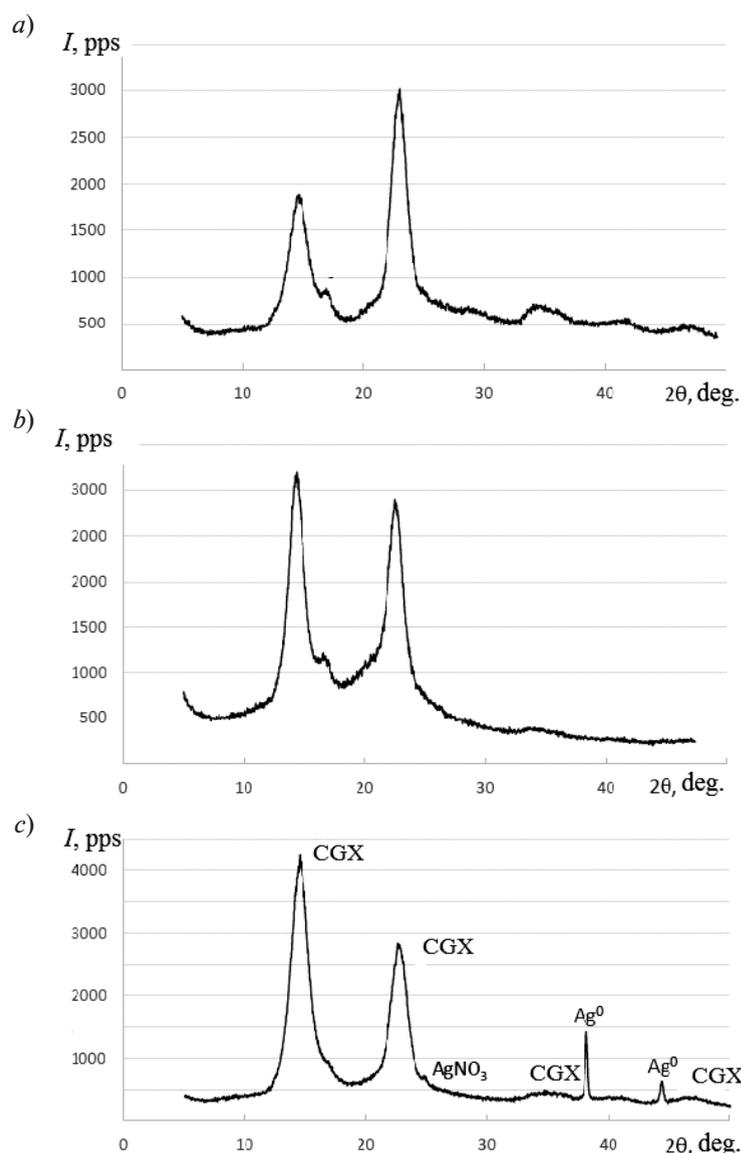


Fig. 2. XRD patterns of GXC films: initial native (a), more morphologically ordered disintegrated (b), treated with an AgNO₃ solution during disintegration (c)

Table 1

Results of energy-dispersive analysis of GXC samples treated with a 1% AgNO₃ solution

GXC film	Chemical composition, at%				Ag/N
	C	N	O	Ag	
Native	72.0	0.3	26.0	1.7	5.7
Disintegrated	54.5	5.5	30.0	10.0	1.8

disintegrated GXC films. Three main X-ray scattering peaks can be observed in both cases, localized at 2θ values equal to 15.0° , 16.6° and 22.4° , which indicates that the morphological structure of cellulose is preserved after the disintegration of the GXC film. The width of the peaks confirms that GXC is partially crystalline. The first intensity peak is narrower and higher (Fig. 2, *b*), which indicates an increased degree of orientational order in the crystalline objects after the disintegration

of the GXC film and proves (as discussed in [7]) that constant stoichiometry is preserved in colloids in aqueous suspensions. The XRD pattern in Fig. 2, *c* shows the regions indicating the presence of silver (Ag⁰) and silver nitrate (AgNO₃) nanoparticles in the native and disintegrated GXC films (disintegration was carried out in the presence of a silver solution). This composition is confirmed by the data of elemental microanalysis (see Table 1) used to obtain averaged comparative results of the

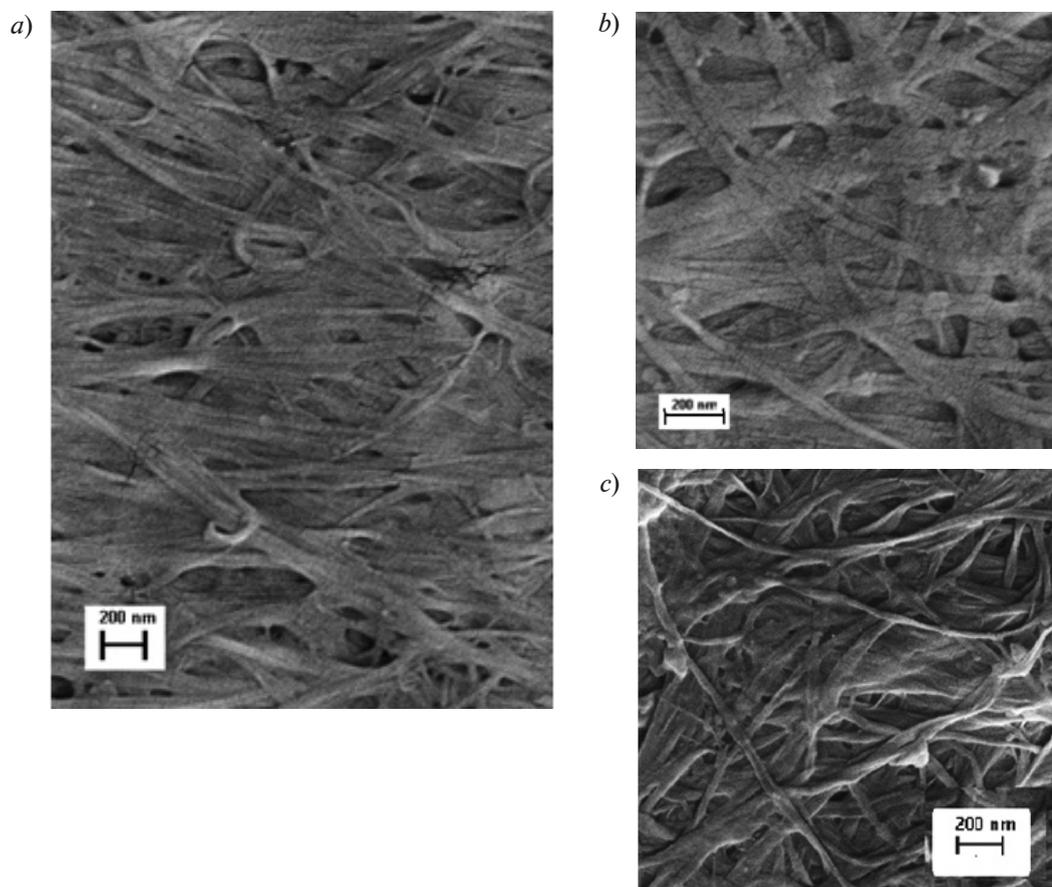


Fig. 3. Micrographs of dried films of native (*a*, *b*) and disintegrated (*c*) GXC obtained by SEM magnified 50,000 (*a*, *c*) and 130,000 (*b*) times

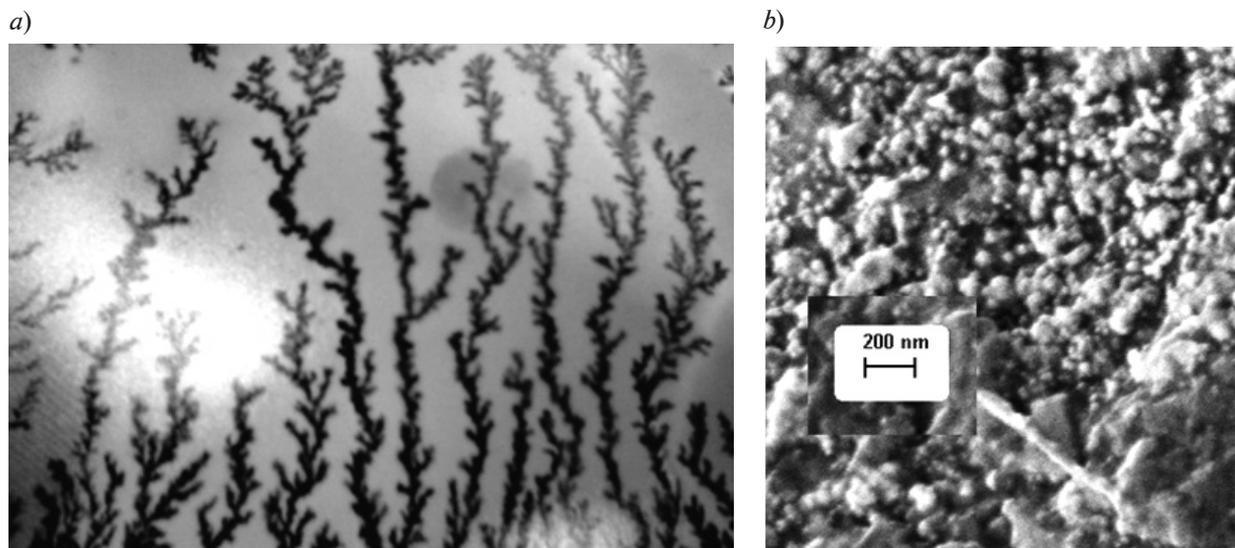


Fig. 4. A photograph (a) and a micrograph obtained by SEM (b) of a dried native GXC film pretreated with a 1 % AgNO_3 solution. Magnification by 1.5 (a) and 50,000 (b) times.

Dendrites of reduced silver in the photograph visualize the elements of the supermolecular structure of the sample

content of chemical elements in native and disintegrated GXC films treated with a 1% AgNO_3 solution.

Fig. 3 shows micrographs of native and disintegrated GXC films obtained by SEM at different magnifications. Characteristic fibril strip structures are visible in Fig. 3, a; alternating crystallite (with the size of about 10 nm) and amorphous regions can be observed in microfibrils at a higher magnification (Fig. 3, b). Such objects of disintegration of GXC films as individual broken strips and chaotically located single macrofibrils are clearly visible in micrographs in Fig. 3, c.

Fig. 4 shows a photograph and a micrograph of a dried native GXC film pretreated with a 1 % AgNO_3 solution. Dendrites of reduced silver visualizing the elements of the morphological nanostructure of GXC can be observed on the surface of the film (Fig. 4, a). Both dendrites and silver nanoparticles (30 – 80 nm) filling the space between the strips are visible on the micrograph (Fig. 4, b).

The situation is different for dried films of aqueous suspensions of disintegrated GXC obtained by introducing a 1% AgNO_3 solution into the blender during disintegration of GXC. Fig. 5 shows a micrograph of such films, where Ag^0 nanoparticles 10 – 50 nm in size, located

in the cavities of the network formed by fragments of disintegrated GXC strips, can be observed to develop. It should be noted that silver nanoparticles form near the elements of the morphological structure of GXC.

The obtained results indicate that both Ag^0 nanoparticles and AgNO_3 salt are contained in

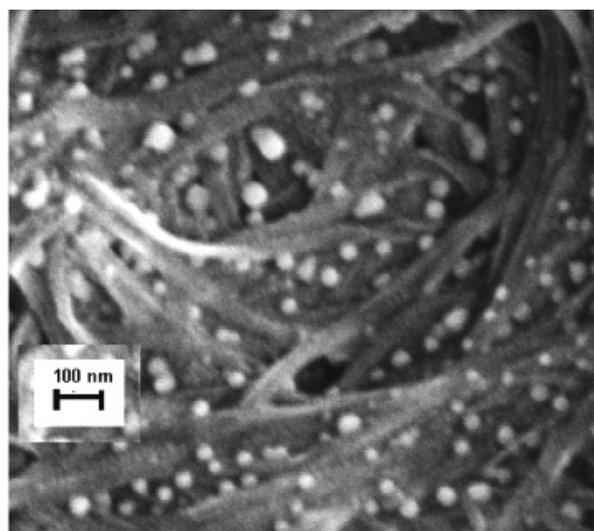


Fig. 5. A micrograph of a GXC film of treated with a 1 % AgNO_3 solution in the process of disintegration. The micrograph was obtained by SEM with a magnification by 100,000 times

samples of dried GXC films treated with a 1 % AgNO_3 solution. The films samples have varying nanoparticle contents. However, it is obvious that the supermolecular structure of GXC is capable of reducing silver atoms from its salt and lead to the appearance of either dendrites from silver atoms in the native GXC or to the formation of isolated silver nanoparticles during the disintegration of GXC in the presence of AgNO_3 solution.

This indicates that chemisorption interactions exist in the cellulose, making it capable of reducing silver atoms near the elements of the morphological structure. Such a result gives further insight into the properties of GXC, for which previously only molecular interactions were known, namely, intra- and intermolecular hydrogen bonds, as well as van der Waals bonds.

Conclusions

We have used scanning electron microscopy and X-ray diffraction analysis to study the effect of mechanical disintegration on the morphology of a native film of *Gluconacetobacter xylinus*

cellulose (GXC) and found that

the morphological structure characteristic for GXC is preserved in the disintegrated films; however, there is a certain change in the orientational order, and a background amorphous phase forms from the disintegrated elements;

silver (Ag^0) nanoparticles and residual silver nitrate are present in the films of native and disintegrated GXC treated with a solution of silver nitrate;

dendrites are formed from reduced silver in dried and solution-treated films of the initial native GXC, which are visible to the naked eye and visualize the morphological structure of GXC;

silver nanoparticles with a predominant size of 10–50 nm, located in the cavities of the GXC network near the structural elements were found in dried GXC films disintegrated in the presence of a 1 % silver nitrate solution.

The chemisorption interactions capable of reducing atomic silver from its salt were discovered in the supramolecular structure of GXC.

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Received 12.12.2017, accepted 14.12.2017.

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