Assessing the impact of lyophilization process in production of implants based on the bacterial cellulose using Raman spectroscopy method

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Abstract. In this article we present the research results of lyophilization process influence on the composition of hybrid materials based on the bacterial cellulose (BC) using Raman spectroscopy method. As an object of research was used BC, as well as hybrids based on it, comprising the various combinations of hydroxyapatite (HAP) and collagen. Our studies showed that during the lyophilization process changes the ratio of the individual components. It was found that for samples hybrid based on BC with addition of HAP occurs increase of $\text{PO}_4^{3-}$ peak intensity in the region $956\,\text{cm}^{-1}$ with decreasing width, which indicates a change in the degree of HAP crystallinity.

Introduction

Bacterial cellulose (BC) is a unique biopolymer, based on which you can solve many problems of medicine. It also used in the food, pulp and paper, electronics, biotechnology and chemical industries. BC, unlike plant-derived cellulose, synthesized by microorganisms in pure form without lignin impurities and other related components [1]. With essentially the same chemical structure as the plant cellulose, BC shows two very important qualities - subtle porosity and mechanical strength. BC is produced by the acetic acid bacteria and it is biodegradable and biocompatible.

According to researches [2,3], faster tissue regeneration and wound closure occurs using biomaterials based on BC compared to bandages. In another experiment [4] a primary effect of BC bandaging material was examined on laboratory rats. It is proved that the presence of BC may promote wound healing due to accumulation of the extracellular matrix.

Using biocompatible biologically active compounds is a method of improving pure BC and allows to get closer to "more ideal" dressing material having improved biocompatibility, antimicrobial properties, and also capable of retaining water [5].

Additional treatment at creation of implants based on BC can be a process of lyophilization (freeze-drying), which can increase the shelf life of biological materials, simplify storage conditions. However, the influence of lyophilization on the structure of implants based on BC are not known.

To investigate the effect of lyophilization process on the component composition of based on BC implants may be used physical methods of control.
In the article [6] Raman spectroscopy method was used to estimate structural differences of celluloses of different origin: produced in the presence of pectin and xyloglucan, as well as commercial cellulose and cellulose extracted from apples. It found that different Raman bands may be used for the successful and rapid determination of the crystallinity degree.

In the article [2] shows the main wavenumbers corresponding to the components of the bacterial cellulose. Thus, a peak at 1095 cm\(^{-1}\) is a sealing ring and stretching vibrations C-O-C of glycosidic bond stretching. In the region 1270-1500 cm\(^{-1}\) bands C-C-H, O-C-H, C-O-H, H-C-H and CH\(_2\) are related to stretching vibrations of cellulose.

The purpose of research was to study the influence of lyophilization process on the composition of hybrid materials based on BC using Raman spectroscopy.

1. Materials and methods of research
In this study bacterial cellulose (BC) has been investigated, as well as hybrids, based on BC, including the different combinations of HAP and collagen: BC and HAP, collagen and BC, BC and HAP with collagen.

To obtain the BC was used the strain Gluconacetobacter sucrofermentas B-11267 [11]. The obtained membrane was washed with distilled water, dried in an oven at 80°C to constant weight. HAP was prepared according to the original method [8].

To prepare composites were added to the solution: 1% HAP (HAP + the BC); 0.5% of collagen (collagen + the BC), 1% HAP and 0.5% collagen (the BC + HAP + collagen). A part of composites further lyophilized using standard method [9].

The spectral characteristics of the materials based on BC were studied using an experimental stand, including the high-resolution digital spectrometer Andor Shamrock sr-303i with built-in cooling camera DV420A-OE, a fiber optic probe for Raman spectroscopy RPB785, combined with laser module LuxxMaster LML-785.0RB-04 (up to 500 mW, wavelength 785 nm) [12]. Separation of Raman spectrum from the background of autofluorescence carried out by means polynomial approximation of a fluorescent component and subtracting it from the recorded spectra.

2. Results and discussion
As a result of studies were obtained spectra of lyophilization and illyophilization types of the bacterial cellulose.

![Figure 1. The normalized by the average value, Raman spectra of lyophilization and illyophilization samples BC](image)
Figure 1 shows that in illyophilization samples in the wavenumber range 1180-1270 cm\(^{-1}\) dominated the curves H-C-C, H-C-O, H-C-H, C-O-H of cellulose compared to with lyophilization samples. These changes are due to the fact that the sample has been dried during the lyophilization. It is also seen that the lyophilization process does not change the main peak 1096 cm\(^{-1}\), which corresponds to the cellulose.

The following figure 2 shows the spectral analysis of various studied hybrids based on BC.

(a)

(b)
Figure 2. Normalized to the average value of the Raman spectra of lyophilization and illaryophilization hybrid based on BC with the addition of (a) collagen, (b) HAP, (c) HAP and collagen

Analysis of figure 2a shows that in the region 1230-1270 cm\(^{-1}\), corresponding to vibrations of Amide III, there is no significant changes during the lyophilization process, which indicates that the lyophilization process has no damaging effect on the collagen matrix in the composition of the BC.

From Figure 2b, it is seen that after lyophilization of samples with HAP, observed the increasing of PO\(_4^{3-}\) peak intensity in the region 956 cm\(^{-1}\) with decreasing width. In the works of authors [7, 10] have shown that the line of symmetric stretching vibration PO\(_4^{3-}\) (\(\nu_1\)) has a high sensitivity to the mineral encirclement: the frequency and form of this line depends on the local surroundings and change as a result of substitution of anionic groups and changes in the degree of crystallinity. The substituted carbonate-apatite B-type phosphate \(\nu_1\) line appears in the range of 955–959 cm\(^{-1}\). In a crystalline unsubstituted hydroxyapatite line \(\nu_1\) shifted to 962–965 cm\(^{-1}\). Finally, at a frequency line 945–950 cm\(^{-1}\) indicates the presence of a disordered lattice phosphate apatite. In general, the phosphate band \(\nu_1\) is a superposition of three components; it usually has an asymmetric shape due to the disordered vibrations contribution of phosphate and unsubstituted hydroxyapatite. It is obvious that process of lyophilization effects on the ratio of these components, apparently resulting to decrease in the proportion of highly crystalline apatite.

Conclusions
Experimentally found features of Raman spectra for various composite implants based on BC. It was found that during the lyophilization process changes the ratio of the individual components. Thus there is an amplification of cellulose line in the normalized spectrum, indicating at reducing the proportion of the individual components. Moreover, for the hybrid based on BC samples supplemented with HAP revealed an increase in the PO\(_4^{3-}\) peak intensity near 956 cm\(^{-1}\) with decreasing width, which indicates a change in the degree of HAP crystallinity.
Acknowledgments
This work was supported by the Ministry of Education and Science of the Russian Federation.

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