Optical methods for evaluating the composition of combined materials based on bacterial cellulose

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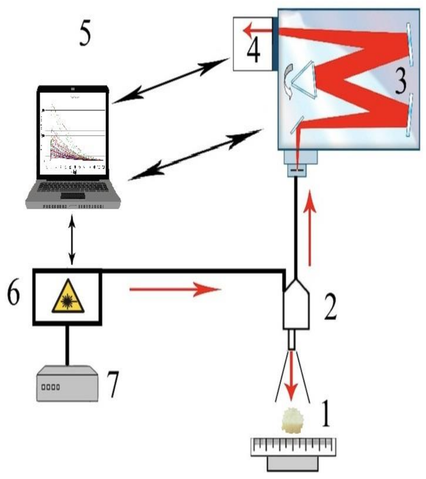
Introduction:

The growing demand for bio-based bacterial cellulose materials has attracted increasing attention for their application in biomedical fields. Bacterial cellulose (BC)-based composite materials promote re-epithelialization, resulting in faster wound healing in regenerative medicine [1]. The engraftment of such biomaterials directly depends on their composition. Therefore, it is an urgent task to control the composition of combined materials based on bacterial biocellulose during their manufacture.

Material and methods of research:

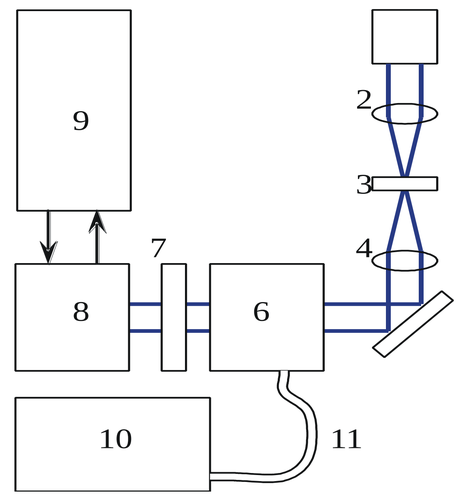
In this work, bacterial cellulose (BC) as well as BC-based combined materials including various additives such as pectin, 1.3-dioctyl-4-methylimidosalic bromide were investigated. Conventionally, all the studied samples were divided into 4 main groups:1- Bacterial cellulose gel with pectin content;2- Bacterial cellulose gel without pectin content;3- 1.3-dioctyl-4-methylimidosaliy bromide bacterial cellulose gel with pectin addition;4-1.3-dioctyl-4-methylimidosaliy bromide bacterial cellulose gel without pectin addition.

To obtain BCs, acetobacteria obtained from Medusomycesgisevii culture by cloning and sowing from 1:100,000 dilution on agarized HS medium (HestrinSchramm, 1954) were cultured. BC gels were washed with running water and placed in 0,1H NaOH solution for 24 hours at room temperature to remove remaining bacterial cells, followed by washing with water, placed in 0,5% HCl solution for 24 hours at room temperature, and stored in 70% ethyl alcohol in plastic dishes at 25-27ºC. Gel-based composites were prepared by synthesizing imidazolium derivatives and soaked in 250 µg/ml DMSO solutions of imidazolium derivatives.



Raman spectroscopy bench 1 - object under study, 2 - Raman probe RPB785, 3 - high resolution digital spectrometer Andor Shamrock sr-303i, 4 - built-in cooled camera DV420A-OE, 5 - computer, 6 - laser module LuxxMaster LML-785.0RB-04, 7 - power supply of laser module

Confocal microscopy bench1 - visible light source (halogen lamp), 2 - collimator, 3 - object, 4 - objective lens, 5 - rotating mirror, 6 - confocal scanning unit, 7 - filter unit, 8 - camera, 9 - computer, 10 - laser unit [2].



Results of research:

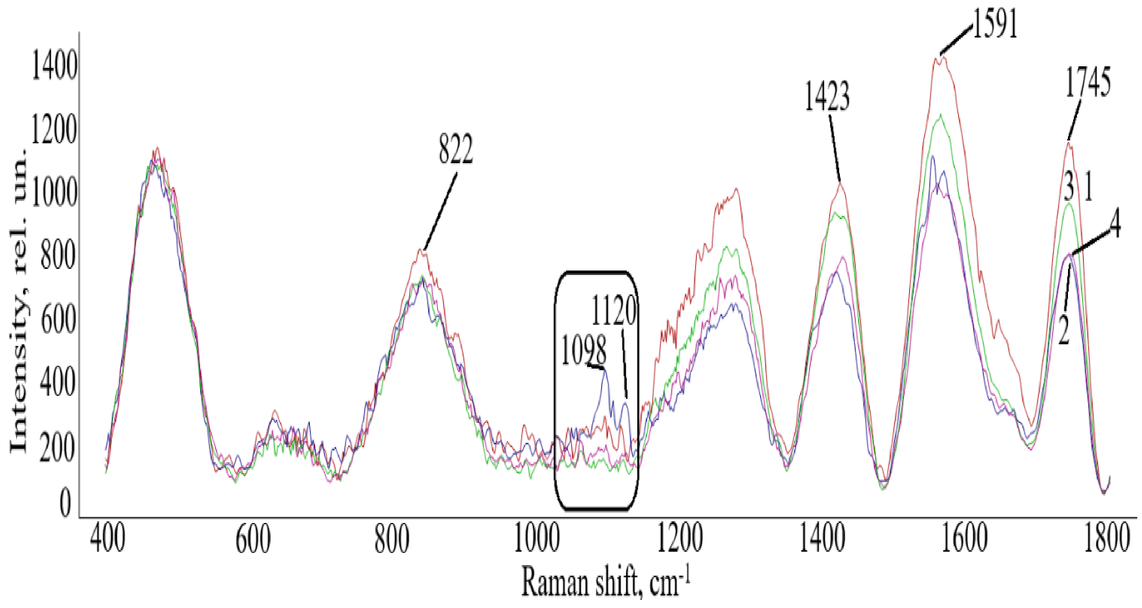


Figure 1. Averaged CR spectra of the studied groups: groups 1 (red line) and 2 (blue line), groups 3 (green line) and 4 (purple line)

The CR lines ~ 1098 cm^{-1}, 1120 cm^{-1}, 1180-1275 cm^{-1}, 1423 cm^{-1} and 1745 cm^{-1}, corresponding to cellulose fluctuations, are most pronounced in samples of bacterial cellulose without pectin addition. The CR line ~ 1098 cm^{-1} is found only in samples of bacterial cellulose without pectin. .

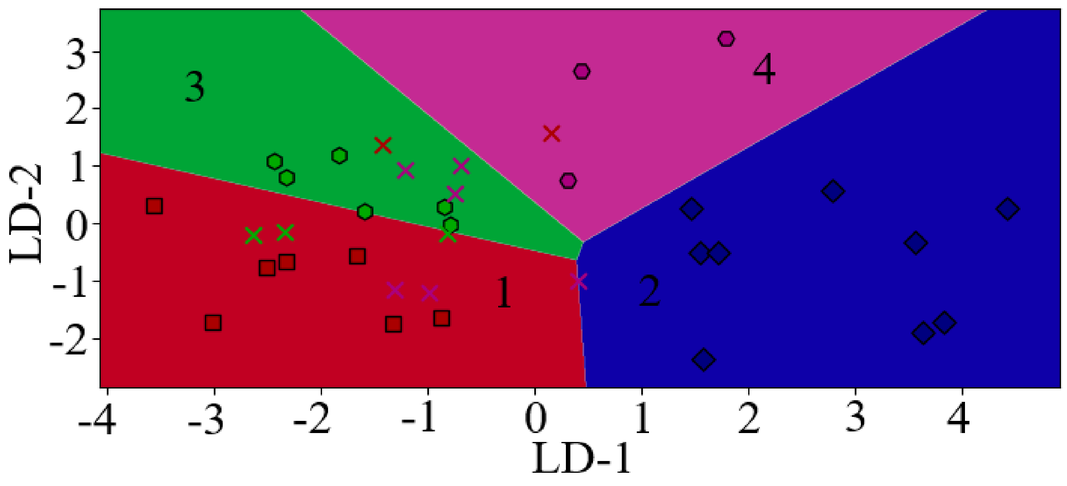


Figure 2. LDA results. Graph of linear discriminant function values(1, 2, 3 and 4 study groups).

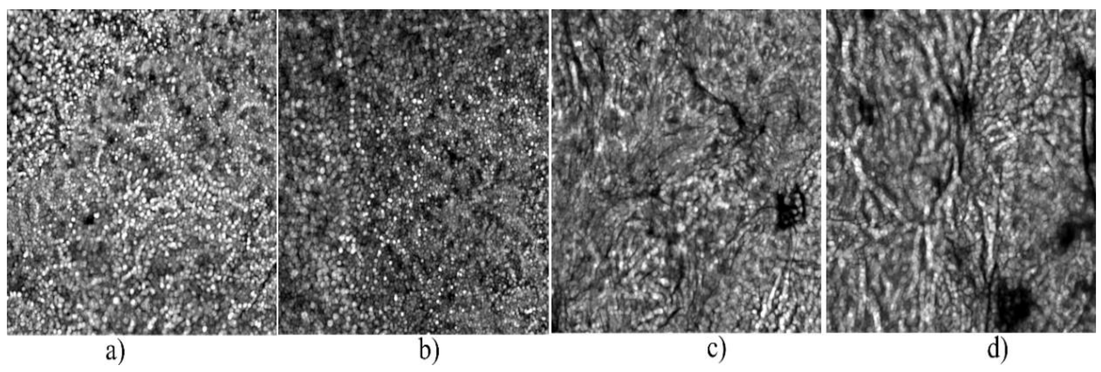


Figure 3. Micrographs of bacterial cellulose (a- group 1, b- group 2, c- group 3, d- group 4) obtained by confocal microscopy. Magnification 400*400 µm

Conclusion:

Using Raman spectroscopy and using LDA, as well as analysis of variance, spectral differences between the studied groups of bacterial cellulose with and without the addition of pectin and with the addition of 1.3-dioctyl-4-methylimidosalic bromide were obtained. It was found that the main differences in the CR spectra appear on the following lines 1745 cm^{-1}, 1591 cm^{-1} and 1092 cm^{-1}, corresponding to the main lines of the bacterial cellulose.

An algorithm for identification of objects based on bacterial cellulose was developed. It was found that if the amplitude of the CR line ~ 1745 cm^{-1} is 0.018 rel.un., it is the spectrum of bacterial cellulose without pectin content, while if the amplitude of the line 1277 cm^{-1} is 0.075 rel.un., it is the spectrum of bacterial cellulose with pectin content.

Thus, using the method of CR spectroscopy it is possible to perform express analysis of the composition of the studied samples based on bacterial cellulose. The microscopic analysis revealed changes in the structure of bacterial cellulose upon addition of pectin in all the studied samples.

References:

1. Raquel Portela, Catarina R. Leal, Pedro L.Almeida and Rita G. Sobral, Microbial Biotechnology, 2019. vol.12(4). p. 586–610. DOI: 10.1111/1751-7915.13392

2.E.V Timchenko, P.E. Timchenko, E.V. Pisareva, M.A. Daniel, L.T. Volova, A.A. Fedotov, O.O. Frolov, A.N. Subatovich, Journal of Optical Technology, 2020, vol.87(3) p. 161-167. DOI: 10.1364/JOT.87.000161