PR-42

WOUND HEALING BACTERIAL CELLULOSE BASED BIOCOMPOSITE MATERIAL WITH CHITOSAN AND *BACILLUS SUBTILIS* EXOMETABOLITES

Savitskaya I. S., **Kistaubayeva A. S., Shokataeva D. H.**
Al-Farabi Kazakh National University, Almaty, Kazakhstan
E-mail: irasava_2006@mail.ru

Abstract. Bacterial cellulose (BC) gel-film is a matrix-carrier of almost any drug and can be used as a universal wound dressing for burns, mechanical and other types of injuries [1].

Protective wound dressings made of chitosan are air and vapor permeable, prevent the invasion of the wound by microorganisms, create an optimal microclimate in the wound, and promote cell proliferation [2].

Bacteria of *Bacillus* genus in the course of their vital activity produce postbiotics - metabolic products that have biological activity in relation to host organism. The production of a wide range of antibiotics and proteolytic enzymes by bacteria of the *Bacillus* genus, stimulating tissue regeneration processes, is the reason for studying the possibility of using metabolites of these bacteria for functionalization of wound dressings based on BC gel film [3].

Aim: development of a wound-healing biocomposite material with antimicrobial properties by including chitosan and *Bacillus subtilis* exometabolites in the BC.

The inclusion of functional ingredients in the composite was carried out by their joint aggregation with the BC gel film. Films of bacterial cellulose were immersed in a solution of chitosan dissolved in an aqueous solution of 1% acetic acid to a concentration of 0.6% and incubated for 6 h at room temperature. The mass ratio of BC and chitosan was 75:25. The films were stored at a relative humidity of 50% and a temperature of 25 °C.

The structural properties of BC and BC/chitosan (BC/Ch) gel films were studied using a Quanta 3D 200i Dual system scanning electron microscope. The composite material BC/Ch has an interconnected porous matrix structure with a large surface area. Micro- (15-35 nm) and macrofibrils (50-150 nm) in BC and BC/Ch films are combined into ribbon-like fibers, providing a high degree of crystallinity (up to 80%) and mechanical strength (Young's modulus: 36.03 ± 1, 80 MPa; tensile strength: 22.48 ± 0.20 MPa). The chemical interaction of BC and chitosan in the composition of the films was established by IR spectrometry.

Separation of native microbial culture (NMC) was carried out by centrifugation and membrane filtration. The presence of fermentation products with biological activity in the cell-free supernatant of the culture fluid (CFSCF) was asessed by three parameters: protein content, proteolytic and antagonistic activity. After 24 hours of cultivation of *B. subtilis* P-2 strain, the CFSCF accumulated up to 0.541±13.4 mg/ml of protein. The level of proteases in the NMC reaches 9.3±0.6 U/ml, and in the supernatant - 7.8 ± 0.3 U/ml, i.e. the level of these enzymes in the supernatant is only 0.8 times lower than in the NMC.

The level of antimicrobial activity of *B. subtilis* P-2 NMC and CFSCF was evaluated by the size of growth inhibition zone of target microorganisms. The antagonistic activity of *B. subtilis* P-2 CFSCF is almost 26% lower than that of the strain NMC. Nevertheless, the level of antagonistic activity of the supernatant remains rather high, which indicates on the presence of antimicrobial substances in it.

The immobilization of metabolites contained in *B. subtilis* P-2 CFSCF was carried out through the adsorption of metabolites on a film of bacterial cellulose. Immobilization of protein molecules occurs after 150 min of incubation. The efficiency of immobilization was 74%. The proteolytic activity of the *B. subtilis* P-2 bacterial culture liquid was 7.8 ± 0.6 U/min x ml.

Preclinical tests of immobilized postbiotic from *Bacillus subtilis* exometabolites on a model of cut wounds, localized purulent infection and thermal burns in experimental animals showed its therapeutic efficacy. The use of the obtained material in treatment of wounds in laboratory animals reduces the healing time by an average of 20%. The therapeutic effect is due to the complex action of the functional ingredients included in biocomposite material: postbiotic in combination with chitosan.

References

1. Lynd L. R., Weimer P. J., van Zyl W. H., Pretorius I. S. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiology and Molecular Biology Reviews. 2002, vol.66, No. 3, p. 506.

2. Lin W. C., Lien C. C., Yeh H. J., Yu C. M., Hsu S. H. Bacterial cellulose and bacterial cellulose-chitosan membranes for wound dressing applications. Carbohydr Polym. 2013, vol. 94, No. 1, pp. 603–611.

3. Smirnov V. V., Reznik S. R., Vyunitskaya V. A. Modern concepts of the mechanisms of therapeutic and prophylactic action of probiotics from bacteria of *Bacillus* genus. Journal of Microbiology. 2002, vol. 24, No. 4, pp. 92–112.

This work was supported by the Ministry of Education and Science of the Republic of Kazakhstan, project # 2679/GF4 «Development of biocomposite materials on the basis of bacterial cellulose for creating the transdermal therapeutic systems».