ABSTRACT
of the Thesis for the Philosophy Doctor (PhD) degree
Specialty "6D070100-Biotechnology»

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Ways of bacterial cellulose application for creation of biocomposite materials with antimicrobial and probiotic properties

General characteristics of the work.

The work is devoted to substantiation of methodological principles for development of technologies for obtaining biocomposite materials based on bacterial cellulose with antimicrobial, wound-healing, probiotic properties and laboratory confirmation of their effectiveness.

Relevance of the topic.

The design features of bacterial cellulose (BC): its micro- and nanofibrillar structure, high porosity, and crystallinity give a huge potential for creating various composite materials based on it. A characteristic structural feature of the BC is that the aggregates of fibrils occupy an insignificant part of the volume, which makes it possible to introduce into BC not only a variety of substances, including those with biological activity, but also cells of prokaryotic or eukaryotic organisms. They give the cellulose carrier matrix new functional properties. An example of such functionalization is the inclusion of substances with antimicrobial activity. Most often, such materials are used to create wound coatings, since the BC itself is tinned only with a mechanical barrier, protecting the wound surface from drying out and absorbing tissue decay products. Creating antimicrobial coatings, the BC gel film includes antibiotics and antiseptics. Unfortunately, by now, many pathogens of wound infections already have multiple antibiotic resistance. There is no such resistance to antiseptics, but they have only antimicrobial activity, without having a wound-healing effect.

In this regard, the wound coating, which includes an agent that, in addition to antimicrobial action, also has an activity that ensures tissue regeneration, may be promising. Such a functional agent can be chitosan, which has antimicrobial activity and promotes tissue regeneration. In this regard, combined films consisting of chitosan, included in BC matrix are becoming popular.

One of the promising areas of modern biotechnology is the development of medicines based on biologically active substances produced by bacteria, including representatives of *Bacillus* genus. They produce a wide range of antibiotics in minimal amounts, and stimulate local and systemic immunity. The proteolytic enzymes synthesized by these bacteria promote tissue regeneration, have a thrombolytic effect, prevent scar formation, and lyse necrotic tissues. A number of studies provide data on successful wound healing therapy using *Bacillus* cultures. This makes it possible to use them for local wound treatment and prevention of purulent complications. The production of a wide range of antibiotics and proteolytic enzymes by *Bacillus* bacteria that stimulate tissue regeneration processes is the reason for the study of the possibility

of using these bacteria for the functionalization of wound coverings based on BC gel-film.

In addition, according to recent studies, the use *of Bacillus* species and especially *B. subtilis* (BS) as a probiotic has gained huge interest. These bacteria are effective in preventing respiratory infections and gastrointestinal disorders, as well as in overcoming symptoms associated with irritable bowel syndrome. Presence of *B. subtilis* promotes the maintenance of a favorable, balanced gut microbiota and enhances the growth and viability of probiotic cells of lactic acid bacteria. It has also been suggested that these probiotic properties are related to its ability to stimulate the immune system and produce antimicrobial substances, or even induce signal interference against pathogens. Such purposeful correction of disturbed biochemical and physiological processes by introducing bacteria that produce biologically active substances is commonly called the "microbial saprophytic pharmacopoeia".

The successful immobilization of bacteria and enzymes on BC with the preservation and even increase of their physiological activity was the basis for conducting this study aimed at creating biocomposite materials with antimicrobial and probiotic properties by including chitosan, cells and exometabolites of bacteria of the *Bacillus* genus in BC.

The aim of the work: Development of a technology for production and functionalization of bacterial cellulose for the creation of composite materials with antimicrobial, wound-healing and probiotic properties.

Tasks of the work:

- select and identify a new active BC producing strain;
- modify the nutrient medium composition based on industrial waste for the BC producer and study the structural properties of biopolymer samples obtained under surface and agitated culture conditions;
- develop a method for producing composite materials based on BC with reinforcing components: chitosan, BS cells or exometabolites;
- determine the biocompatibility of biocomposite materials *in vitro;*
- determine the antimicrobial activity of biocomposite materials;
- determine the wound-healing activity of biocomposite materials;
- study the effect of immobilization of bacilli in BC granules on their probiotic properties.

Research objects: Gel films and BC globules, as well as experimental samples of biocomposites with chitosan and BS cells/exometabolites included in its composition.

Subject of the study: Determination of structural features, antagonistic, wound-healing and probiotic activity of biocomposite materials based on BC matrix for the subsequent development of transdermal and probiotic therapeutic systems.

Research methods: BC production – surface and submerged culture; biocomposites - adsorption and spatial immobilization. Structure of films and biocomposites – scanning electron microscopy. Films strength - breaking machine "Instron". IR spectrum – Fourier transform spectrometer FSM-1201. Antagonistic activity – agar diffusion and Time-kill test. Biocompatibility - viability, adhesive properties, metabolic activity, morphology and proliferation of NIH3T3 murine fibroblast cells. Wound-healing efficacy – a model of a cut wound in experimental

animals. Probiotic effect – antibiotic-induced dysbiosis.

Scientific novelty of the research results.

A new producer strain with a high level of BC biosynthesis was obtained. Cultivation conditions are selected and optimized to ensure maximum yield of the target product.

For the first time, bioactive wound coatings based on BC gel film with immobilized *BS* cells and metabiotics that have proliferative antiseptic, necrolytic, and antioxidant effects have been developed.

For the first time, probiotic BC/*BS* microgranules, intended for correction and restoration of the intestinal microbiome have been obtained.

Theoretical significance of the work. The choice of composition of composite materials with antimicrobial and probiotic effects is theoretically and experimentally justified. The choice of the type of sorbent used for immobilization of spore bacteria and their metabolites is justified.

The ways of a cost-effective method for obtaining BC and its functionalization with biologically active components for obtaining biocompatible composite materials with high antimicrobial, wound-healing and probiotic activity are indicated.

Possible mechanisms of wound healing and probiotic action of the microbiological basis of biocomposites – *BS* bacteria – are presented.

The practical value of the study. An optimized culture medium based on sugar production waste – molasses, the *Komagataeibacter xylinus* C-3 strain and the technological conditions for its cultivation can be used for industrial production of BC.

Wound coatings made of BC impregnated with chitosan, *Bacillus* cells and/or exometabolites can be used as elements of a new sorption dressing.

Probiotic BC/BS microgranules can be used to correct the large intestine microbiocenosis.

Basic statements for the defense:

- a new medium with molasses, the cultivation of the producer on which provides a high level of BC biosynthesis, is cost-effective;
- the “adsorption-incubation” method used for surface and spatial immobilization of BS ensures a high concentration of cells per unit volume of the carrier both on the surface and inside it;
- the efficiency of cell immobilization depends on matrix parameters such as surface area and total porosity, which can be increased by alkaline treatment of BC and the addition of porogens during its biosynthesis;
- one of the ways to use BC is to create a biocomposite material with antimicrobial and wound-healing activity by including of *BS* cells in the BC matrix;
- the sorbent carrier can include not only live bacterial cells, but also their biologically active metabolites with antimicrobial and proteolytic activity;
- spatial immobilization of cells in BC granules increases their viability and enhances probiotic properties, allowing for effective correction of intestinal dysbiosis.

The main research results and conclusions:

Complex research on the creation of new materials with antimicrobial and probiotic properties by including functional ingredients in the BC carrier matrix was conducted. As a result biocomposite materials based on bacterial cellulose,

chitosan, cells and metabolites of BS were obtained and characterized . The use of these composites in the treatment of experimental conditionally clean incised wounds in animals stimulates reparative processes and reduces the healing time. It was shown that the use of probiotic microgranules of BC/BS normalizes the intestinal microecology of experimental animals. Possible mechanisms of wound-healing and probiotic action of the obtained materials are presented.

As the result of research, the following conclusions can be drawn:

1. A new producer of bacterial cellulose was selected, which in terms of productivity exceeds the collection strains recommended for its industrial production. The maximum yield of BC on a standard HS medium under surface cultivation conditions is 4.6 g/l and 6.7 g/l under submerged cultivation conditions.

Based on a combination of morphological, cultural, physiological properties and molecular genetic analysis, it was determined that it belongs to the *K. xylinus* species.

2. The optimal composition of molasses-based nutrient medium for BC producer has been developed, which provides a 5-fold reduction in the cost of the biopolymer synthesized by it. Surface cultivation of the *Komagataeibacter xylinus* C3 strain on a medium with molasses for 7 days increases the productivity of film formation to 12.8 g/l. The introduction of 0.04% NaAlg into the medium for submerged cultivation leads to the formation of up to 12.1 g/l BC in the form of homogeneous small granules with a size of 130-140 microns. BC obtained on a medium with molasses forms a network of micro - (15-35 nm) and macrofibrils (50-150 nm), providing a high degree of mechanical strength (tensile strength-37.12$\pm$0.2Mpa; elongation at break-3.28$\pm$0.2 %).

3. Three types of biocomposite materials were obtained: BC/BS cells; BC/chitosan; BC/chitosan/BS exometabolites. The inclusion of functional ingredients in composites composition was carried out by their joint aggregation with a BC gel film or globules. The developed technology for producing biocomposites ensures the content of up to 10^8 viable *B.subtilis* bacterial cells in 1 g of film and lyophilized globules.

4. The biocompatibility of the studied composites was determined *in vitro*. Materials containing chitosan and *BS* metabolites did not affect the cytological and proliferative characteristics of mouse NIH3T3 fibroblasts. Cells cultured on a medium with materials were characterized by a high level of metabolic activity in the MTT test (>85%), viability in the trypan blue exclusion test (>90.1%) and LDH test (>85.2%), which indicated the absence of cytotoxicity.

5. BC/BS and BC/Ch/MBS gel films showed maximum bactericidal activity (100% bacterial death) after 24 h for gram-negative and 10 h for gram-positive bacteria. Chitosan-reinforced BC gel films with *BS* metabiotics had the highest antagonistic activity against pathogens of wound infections: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*.

6. The use of the obtained materials in treatment of experimental conditionally clean incised wounds in laboratory animals stimulates reparative processes and reduces the healing time by an average of 24%. The biocomposite BC/Chitosan/*B. subtilis* exometabolites has the highest therapeutic efficacy on the used model of the wound process.

7. Immobilization of the *B.subtilis* antagonist cells into globules resulted in a probiotic biocomposite containing up to 10^8 microbial cells per 1 g. The sorbed probiotic restores the intestinal normoflora of laboratory animals with antibiotic-induced experimental dysbiosis with absolute reduction and elimination of opportunistic microorganisms from the intestine. The inclusion of *B.subtilis* in the BC matrix provides their increased resistance to the bactericidal action of gastric juice.

Thus, all the tasks set in the dissertation have been completed.

Connection with the plan of basic scientific works.

The dissertation work was carried out within the framework of the project 2679 / GF4 «Development of biocomposite materials based on bacterial cellulose for creating transdermal therapeutic systems»

Approbation of the work.

The materials of the dissertation were reported and discussed at the following conferences:

- Scientific conference of students and young scientists "Farabi Alemi" (April 14-16, 2015, Almaty);
- European Biotechnology Congress (7-9 May 2015, Bucharest, Romania);
- 6th International Weigl Conference on Microbiology (8-10 July 2015, Gdansk, Poland);
- International Conference on Advances in Management Science and Engineering (AMSE2015) (26-27 July 2015, Phuket, Thailand);
- III International Farabi Readings. International scientific and practical work conference "Modern problems of biotechnology: from laboratory research to production" (Almaty, 2016);
- International scientific and practical work conference "Contribution of microbiology and virology to the modern bioindustry", dedicated to 60th anniversary of the Institute of Microbiology and Virology (Almaty, 2016);
- 41st FEBS Congress on Molecular and Systems Biology for a Better Life (03-08 September 2016, Kusadasi, Turkey).
- IV International Farabi Readings. International Scientific conferences of students and young scientists "Farabi Alemi" (April 10-11, 2017, Almaty);
- V International Farabi Readings. International scientific conferences of students and young scientists "Farabi Alemi" (April 10-11, 2018, Almaty);
- X International Symposium «The physics and chemistry of carbon and nanoenergetic materials» (12 September 2018, Almaty);
- XV International Scientific and Practical Ecological Conference "Biological species in the structural and functional hierarchy of the biosphere "(October 8-12, 2018, Belgorod, Russia).
- The 5th Symposium on EuroAsian Biodiversity (1-3 July, 2021, Mugla, Turkey; Almaty, Kazakhstan);
- III International Symposium (27–28 May 2021, Belgorod, Russia);
- International Conference on Microbiology “Biology and Biotechnology of Microorganisms ICMBB 2021” (September 16-17 2021, Tashkent, Uzbekistan).

Publications.

On materials of the thesis were published 24 works, including 3 articles in peer-reviewed international scientific journals indexed in the Web of Science or Scopus databases with non-zero impact factor; 1 article in international peer-reviewed scientific journal, (Q1) according JCR in Web of Science Core Collection and having in the database Scopus index percentile at CiteScore not less than 35; 2 articles in international peer-reviewed scientific journals included in the Q3 according JCR in Web of Science Core Collection; 3 articles in national journals from the list of Committee on control in education and science; 13 abstracts in the proceedings of international conferences; 1 patent, 1 patent application of the Republic of Kazakhstan. The contribution to the creation of articles is to perform work on the systematization of the material, analysis of the research results and preparation of data, as well as writing the text of articles. The percentage of author's participation is about 50%.

The volume and structure of the dissertation.

The dissertation is presented on 117 pages of computer text and consists of the following sections: notations and abbreviations, introduction, literature review, materials and methods, research results and their discussion, conclusion, references used from 243 sources. The work includes 18 tables, 45 figures and 2 appendices.

Compliance with the directions of scientific development or state programs: rational use of natural resources, including water resources, geology, processing, new materials and technologies, safe products and structures.