EFFECT OF BIOCOMPOSITES ON BACTERIAL CELLULOSE-BASED HYDROGEL AND PHYSIOLOGICALLY ACTIVE COMPOUNDS ON REGENERATION PROCESSES IN THE SKIN'S LIPID PHASE AFTER BURN INJURY

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Abstract. The article considers the development of biocomposite materials as bacterial cellulose-based hydrogel, chitosan and alginate, plus physiologically active compounds – fusidic acid, resveratrol and dihydroquercetin. It has been found that the use of hydrogel systems derived from microbial polysaccharides and containing bacterial cellulose (BC) / sodium alginate with CaCl2 /sodium fusidate (SF) and BC/chitosan /sodium fusidate helps to reduce the intensity of lipid peroxidation processes and stabilize phospholipid and fatty acid composition of the skin. It is consistent with the findings of the authors on longer release of sodium fusidate from biocomposite materials of this composition. Therefore, the use of BC and chitosan in combination with sodium fusidate, which exhibits antibacterial properties, and the cross-linking of sodium alginate with calcium chloride solution proves to be the most effective for restoring the skin's lipid composition and shortening the course of treatment. Most likely, this effect must be explained by the constant release of physiologically active compounds from hydrogel composites and its impact on damaged skin areas.

Keywords: bacterial cellulose, hydrogel, polysaccharides, fusidic acid, resveratrol, dihydroquercetin, lipids, burn.

Introduction

Cellulose, as one of the most common biodegradable materials in nature, is traditionally synthesised from plants, but it can also be produced by certain types of bacteria (Revin *et al.*, 2020). Bacterial cellulose (BC) mainly produced by microorganisms of the genus *Komagataeibacter* has a unique nanoscale network structure. That is why it is often called bacterial nanocellulose and classified as nanocellulose materials (Aljohani *et al.*, 2018; Ahmed *et al.*, 2019; Anton-Sales *et al.*, 2019; Alven & Aderibigbe, 2020; Cabañas Romero *et al.*, 2020; Ullah *et al.*, 2021; Lunardi *et al.*, 2021; Randhawa *et al.*, 2022; Revin *et al.*, 2022).

Based on this, in recent decades, the use of BC as a biomaterial for tissue engineering, the creation of wound dressing and controlled drug delivery has been of great interest to the researchers (Campano *et al.*, 2016; Aljohani *et al.*, 2018; Demetgül 2018; Anton-Sales *et al.*, 2019; Carvalho *et al.*, 2019; Cazón *et al.*, 2019; Dutta *et al.*, 2019; Alven & Aderibigbe, 2020; Swingler *et al.*, 2021; Choi *et al.*, 2022; Chen *et al.*, 2022; Kushwaha *et al.*, 2022; Khan *et al.*, 2022; Moradpoor *et al.*, 2022). The prospects for using BC as a wound dressing are strengthened by its ability to provide gas exchange, absorb wound effluent, and maintain a moist environment that enhances re-epithelialisation and to serve as a physical barrier against bacterial infections (Anton-Sales *et al.*, 2019).

There are quite a few findings in the research literature that cellulose membranes, plant or bacterial, are clinically safe for the treatment of a third-degree burns in rats (Ahmed *et al.*, 2019). Apart from that, wet dressings made of BC, in particular Biofill® and XCell®, can be successfully used to treat various skin lesions, such as basal cell carcinoma, burns, chronic ulcers, because to their tight grip to the wound, spontaneous undermining after re-epithelialization and ability to shorten the recovery time of damaged tissue (Anton-Sales *et al.*, 2019). Wound healing is a biochemical process complicated by extensive infection caused by various pathogenic microorganisms. Given this, it is of current concern to create BC-based biocomposites with antibacterial, anti-inflammatory and regenerating effects.

A great deal of attention has recently been drawn to the use of bacterial cellulose-based hydrogel as they are able to create an optimal microenvironment for wound healing, high absorption properties in respect to wound effluent, prevention of penetration of microorganisms, as well as high elasticity, absence of antigenic and allergic effects. In addition, in order to ensure a constant influx of biologically active substances and the effectiveness of the use of BC-based wound coverings, it is possible to include various substances in its composition, in particular, biocompatible polysaccharides, antibiotics and antioxidants.

It is known from the literature review that chitosan and alginate produce antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*, have wound healing effects and accelerate epithelialisation (Kwa *et al.,* 2020; Lahiri *et al.,* 2021). Fusidic acid is an antibiotic produced from the fungus *Fusidium coccineum*, belonging to the class of steroids, but without a corticosteroid effect. It has high antibiotic activity against *Staphylococcus aureus* and can be used as part of a wound dressing for burns. The use of naturally occurring antioxidants, resveratrol and dihydroquercetin, is also important for the improving the wound healing after burns because of their ability to impair the intensity of lipid peroxidation when cell membranes are damaged, as well as to demonstrate antibacterial activity against *S. aureus* (Shevelev *et al.*, 2018). It is widely recognised that lipids largely influence the course of pathological processes, since they are characterized by high lability, and the products of lipid metabolism cause destructive changes in cell membranes. In particular, lysophospholipids regulate the activity of most membrane-bound enzymes, the development of physiological reactions and pathological processes (Liu *et al.,* 2021). Ceramides also act as an active secondary messenger, regulate the proliferation and differentiation of keratinocytes, enhance the production of pro-inflammatory cytokines and modulate immune responses (Li *et al.,* 2019).

A great deal of attention is paid to phospholipids and fatty acids, ensuring their diversity, and having an impact on fundamental processes of body regulation: ionic homeostasis, the functioning of most regulatory proteins, gene transcription and synthesis of various highly active lipid bioregulators. Thus, a reduction in expression of elongase, which is involved in the elongation of fatty acids, suppresses the synthesis of these acids, leading to changes in the skin's lipid composition and, therefore, can affect the skin's performance of its functions (Cui *et al.,* 2016).

Based on this data, the purpose of this work is to study the effect of biocomposites of various compositions presented by bacterial cellulose hydrogel on regeneration processes in the lipid phase of the skin after burn.

To achieve this purpose, the following research areas were defined:

– To look into the changes in the quantitative content of phospholipids, fatty acids composition of the lipid phase of the skin and lipid peroxidation products after burn.

– To develop biocomposite materials based on bacterial cellulose hydrogel, biocompatible polysaccharides of chitosan and alginate, fusidic acid, resveratrol and dihydroquercetin and to make a comparative analysis of their effect on changes in lipid composition and the content of their peroxidation products in the skin after burn injury.

Materials and Methods

Producing BC

BC was prepared from a strain of the bacterium *Komagataeibacter sucrofermentans* H-110, which was isolated from kombucha, identified through the 16S rRNA analysis and deposited in the Russian Collection of Industrial Microorganisms as number *K. sucrofermentans* VKPM B-11267 (Revin *et al.,* 2020). To prepare BC, a Hestrin - Schramm medium of the following composition was used, g/l: glucose – 20.0; peptone – 5.0; yeast extract – 5.0; sodium hydrophosphate – 2.7; citric acid – 1.15. pH of the medium – 6.0. A medium with molasses at a concentration of 50 g/l, pH 4.5 was also used. The inoculum was prepared in the ES-20/60 shaker incubator (BIOSAN, Latvia) at a stirring speed of 250 rpm and a temperature of 28 °C for one day. The containers with 200 μL of the medium were inoculated in an amount of 10%

of the volume of the medium and cultivated under static conditions at a temperature of 28 °C for six days.

Isolation and purification of BC

The resulting BC was treated three times with 0.1 H of NaOH solution at 80 °C for 30 minutes to remove the cells and components of the medium. To remove the alkali solution, cellulose was cleaned with 0.5% acetic acid solution and distilled water until a neutral reaction. The treatment was repeated 3 times.

The amount of polysaccharide was determined by the weight method, by heating to a constant mass at a temperature of 60 °C using the II accuracy class scales.

Preparation of BC-based hydroges and biocompatible polysaccharides

To obtain a hydrogel, the purified gel film of bacterial cellulose was mechanically grinded by laboratory homogenizer for 10 minutes. Then BC hydrogel with a hydromodule in a ratio of 1:3 was obtained.

Hydrogel of the following composition was obtained: BC/dihydroquercetin, BC /resveratrol, BC/chitosan, BC/chitosan/sodium fusidate (SF), BC/sodium alginate/sodium fusidate and BC/sodium alginate with $CaCl_2$/sodium fusidate. Low molecular weight chitosan (Sigma-Aldrich, USA) was employed for the research. Hydrogel was obtained by mixing a 2% chitosan solution in acetic acid and BC in 1:1 ratio. Dihydroquercetin was added in the amount of 2% to the weight of the hydromodule. Resveratrol was added in the amount of 0.5% to weight of the hydromodule. A 2% solution was prepared from sodium alginate and added to BC in 1:4 ratio. To obtain an antibacterial wound dressing, fusidic acid was introduced into the composite in the form of sodium fusidate in the amount of 7.5 mg per 1 ml of hydrogel. The hydrogel with sodium alginate was kept in a 5% solution of calcium chloride to give shape.

Fourier-transform IR spectroscopy (FTIR spectroscopy)

FTIR spectra were collected on IR-spectrometer IRPrestige-21 (Shimadzu, Japan) in the middle infrared region of 4000 - 400 cm^{-1}. As samples we used bacterial cellulose gel film purified and lyophilised by FreeZone apparatus (Labconco, USA), as well as the hydrogel that have undergone preliminary sample preparation. To prepare the samples, they were grinded with KBr (2 mg of BC per 100 mg of KBr) and pressed into tablets. The spectrum of pure KBr was subtracted from the obtained spectra.

Producing composite materials

Composite materials in the form of wound-healing dressings were fabricated from the obtained hydrogel. To do this, 200 microgels of hydrogel were applied to a 30-mm diameter polymer-based patch and distributed evenly in the centre of the sample. The obtained composites were left for a day at an air temperature of (20 ± 5) °C with a relative humidity of (65 ± 15) %.

Defining the antibacterial activity of biocomposites

The antibacterial activity of the composites was revealed by the disk diffusion in respect to *Staphylococcus aureus*. The lawns of the test microorganism (1×10^5 CFU/plate) were incubated on the medium No. 1 GRM for cultivation of bacteria. In the experiment with hydrogel, a sample well was previously made in the agar, where 200 μL of the sample was put. The plates were put in a thermostat at 37 °C for 24 hours. Then the delayed growth zones of test culture were measured by defining the inhibitory effect of the tested samples on bacterial growth. The delayed growth zone was measured from the edge of the sample to the beginning of the bacterial growth zone on each side of the sample with an accuracy of 1 mm.

Defining the duration of release of the active substance from biocomposite materials

The method relies on the ability of substances to be diffused in liquid medium. To find the desideratum, the composites were dispensed in 100 ml of sterile distilled water for an hour. When the time was over, the sample was taken to a fresh water, and 100 ml of liquid was taken from the test flask and introduced into a 11-mm diameter well with an agarized medium

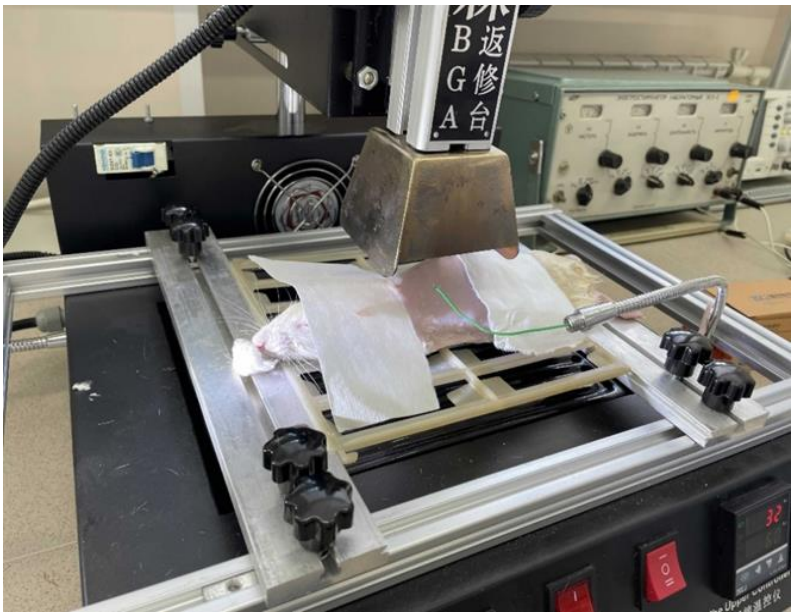

Fig. 1. Causing burn injury in laboratory animals

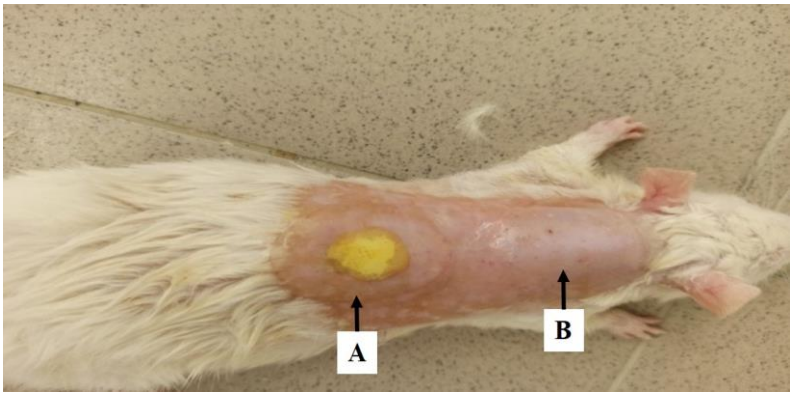

Fig. 2. Application of wound dressing to rats' burn injuries: A – The obtained biocomposite material with hydrogel (BC / dihydroquercetin); B – An adhesive patch (control burn injury)

on a Petri dish previously inoculated with *S. aureus*. The aforementioned action was repeated every hour for 6 hours. The Petri dishes were put in a thermostat at 37 °C for 24 hours, after which the diameter of the delayed growth zone of the test culture was measured considering the size of the well for better clarity of the results.

The object of research and experimentation

To model a pathology, white laboratory rats weighing no more than 300 g were taken to receive burn injuries under zoletil-xylanite anesthesia after exposure to an infrared soldering station Ly M770 for BGA reballing. To cause a burn injury in rats, the fur was removed in the area between the shoulder blades, before any further actions, this place was treated with 70% ethanol. The animals were fixed to a worktable at 15 mm distance from the IR emitter (Fig. 1).

The temperature on the skin of the animals was controlled by a K-type thermocouple and did not exceed 70 °C to develop a wet necrosis. The exposure to IR station continued until the temperature of the animal's skin reached 60 °C,

after which another 30 seconds followed and the station was turned off. Two burns were elicited in each animal. After that, a self-adhesive patch without any active substances was stuck to one of the burns (a control burn), and the other one received a 30-mm diameter patch with the obtained composite material (Fig. 2).

After 7, 14 and 21 days, the biocomposites were being replaced with new ones. Also, after each of these days one animal was killed for the sections of burn injuries to be taken off with a part of healthy tissues for further analysis of the skin's lipid composition.

Ethical approval

All research stages were in compliance with the principles of World Medical Association Declaration of Helsinki (WMA Declaration of Helsinki). The research was also approved by the Local Ethics Committee of Mordovia State University (Protocol № 89 issued on September 12, 2021).

Methods of analysis of skin's lipid fraction after burn injury

The lipids were extracted from nervous tissue using the Bligh-Dyer method (Bligh & Dyer, 1959). To analyse phospholipids (PHL), one-dimensional chromatography was employed together with a solvent system: chloroform/methanol/water/ammonia (60/34/4/2) (Handloser *et al.,* 2008). The lipids were separated using thin-layer chromatography on HPTLC Silica gel 60 F254 glass plates (Merck, Germany). Separate lipid fractions were identified against Rf values, and by utilizing specific staining agents and markers (Supelco).

The quantitative estimation of the lipids was made by the densitometric method on the automated CAMAG TLC Scanner 4 complex (Switzerland). The content of phospholipids (PHL) was presented by the ratio of the inorganic phosphorus of single PHL fractions to the total inorganic phosphorus of all PHL fractions. Fatty acid methylation (FAs) was performed after the Morrison and Smith method (Morrison & Smith, 1964). Quantitative analysis was made by the method of internal standard, where margarine acid was used. The FA methyl esters were separated on a SHIMADZU GC-2010Plus AF gas chromatograph (Japan). The content of diene conjugates (DC) (primary products of lipid peroxidation) was defined by spectrophotometric method at 233 nm; the content of ketodienes and conjugated trienes (secondary products of lipid peroxidation) at 535 nm (Vladimirov *et al.,* 1972).

Results

One of the widely used natural polysaccharides is bacterial cellulose. Due to its structure, it is able to ensure gas permeability, but at the same time it can serve as a barrier for microorganisms, absorb effluents, can be saturated with medication and deliver them to the damaged area. However, BC is rarely used in its pure form. To improve the efficiency of BC applications, biocomposite materials with fundamentally new properties are synthesised on its basis.

Thus, in some earlier researches it was claimed that chitosan could penetrate into the BC pores, forming a denser and more compact matrix structure, as well as impart antimicrobial properties to the biocomposite (Cazón *et al.,* 2019).

Given this fact, at the first stage of research, BC gel films were obtained by utilising the producer strain *Komagataeibacter sucrofermentans* B-11267 on a standard HS medium and molasses medium (Fig. 3).

The polymer yield on the molasses medium exceeds the polymer yield on the standard medium and is 2.19 g/l. The amount of BC on the HS medium was 1.57 g/l (Fig. 4).

The increase in polymer yield is attributed to the fact that in the HS medium glucose as the only carbon source is used not only as an energy source, but also as a precursor of cellulose. In the course of its life, it turns into ketogluconic acids, which significantly reduces the formation of cellulose. In addition, molasses is rich in proteins and organic nitrogen, and it contains a significant amount of sulfur in the form of sulfides and dioxides. The presence of these components in the nutrient medium increases bacteria growth rate.

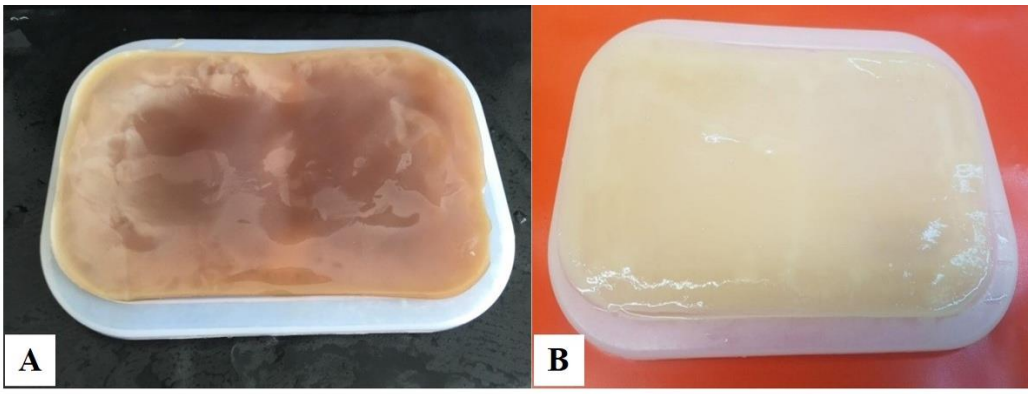

Fig. 3. BC gel film obtained on molasses medium (A) and HS medium (B)

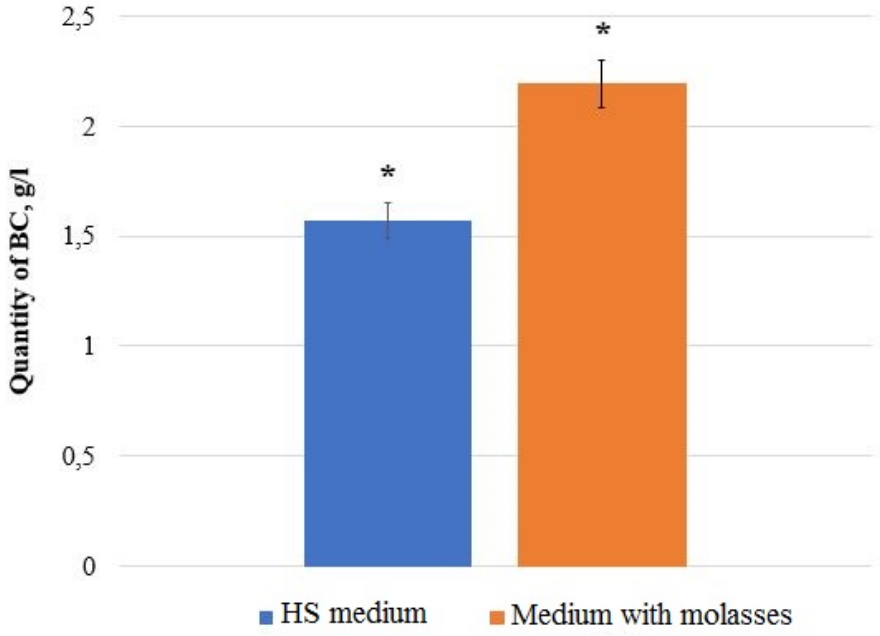

Fig. 4. BC yield when cultivating producer strain *K. sucrofermentans* B-11267 on standard HS medium and molasses medium under static conditions (* – $P \leq 0.05$)

The next stage of research was related with creation of BC hydrogel based on biocompatible polysaccharides with the addition of an antibacterial agent, as using bacterial cellulose as wound dressing requires a certain modification. The hydrogel was prepared by mixing the necessary components with BC hydraulic module.

The following types of hydrogels served as experimental samples: BC/ chitosan, BC/ chitosan /sodium fusidate, BC/sodium alginate/sodium fusidate, BC/sodium alginate with $CaCl_2$ /sodium fusidate, BC/ resveratrol and BC/dihydroquercetin.

Fusidic acid is widely used for systemic and topical treatment of staphylococcal infections, including coagulase-negative staphylococcus and strains resistant to penicillin and other antimicrobial drugs. It is also effective against anaerobic gram-positive strains and shows activity *in vitro* against *Neisseria spp., Bordetella pertussis* and *Moraxella catarrhalis*, but ineffective against other aerobic gram-negative species. *S. aureus* was chosen as a test microorganism because there were enough evidences that this culture is a key pathogen that infects the wound. Also, the uprise of its resistance poses a big

problem for healthcare (He *et al.*, 2021), therefore, the creation of materials that would produce an inhibitory effect on *S. aureus* is essential. In addition, as previously stated, chitosan, resveratrol and dihydroquercetin also have antibacterial activity against *S. aureus* (Cabanas Romero *et al.*, 2020; Shevelev *et al.*, 2018).

Hydrogel was put into the well on a Petri dish with nutrient medium No. 1 GRM, pre-inoculated with S. aureus in the amount of 200 μL. After 24-hour culturing in the thermostat, the microorganism no-growth zone was determined. Table 1 and Figure 5 show the results of the experiment.

Table 1

Antibacterial properties of the obtained materials against *S. aureus*

Sample	No-growth zone, mm
BC/SF	19.5 ± 1
BC/ chitosan	2 ± 1
BC/chitosan/SF	14 ± 1
BC/ resveratrol	6 ± 1
BC/sodium alginate/SF	19.5 ± 1
BC/sodium alginate with $CaCl_2$ /SF	18 ± 1
BC/dihydroquercetin	8.5 ± 1

According to the findings, the antibacterial activity of BC/SF hydrogel and related materials increases because of the introduction of an antibiotic against, for example, that of with BC/chitosan. The differences in antimicrobial activity of BC/SF and BC/chitosan/SF hydrogels can be explained by the emerging amide bonds between chitosan and SF.

The work (Shevelev *et al.*, 2020) claims about a higher activity of resveratrol against *S. aureus* was, but it is not yet confirmed. Since the antibacterial activity of BC/dihydroquercetin had turned out to be greater than that of BC/resveratrol, it was logical to use dihydroquercetin for further research.

The obtained samples were analyzed by means of FTIR spectroscopy.

BC/sodium alginate with $CaCl_2$ /SF hydrogel (Fig. 6) has peaks characteristic of sodium alginate, namely peaks in the region of 1622 and 1430 cm^{-1}. In addition, the presence of SF in the composition is confirmed by peaks at 1740 and 1278 cm^{-1} (Zhang *et al.*, 2017).

Figure 7 shows the peaks characteristic of all the above-mentioned substances. The BC/chitosan spectrum has peaks at 3373 cm^{-1} (overlapping stretch vibrations between O-H and N-H groups), 2930 and 2890 cm^{-1} (C-H stretching), 1633 and 1521 cm^{-1} (amide II band due to C-O and N-H stretching, respectively), 1052 cm^{-1} (skeletal/chain vibration involving C-O stretching) (Demetgül *et al.*, 2018).

The BC/chitosan/SF spectrum has a peak at 1278 cm^{-1}, confirming the presence of SF in the hydrogel composition, as well as peaks 3373, 2930, 1633, 1521 and 1052 cm^{-1} corresponding to the above-mentioned functional groups.

Dihydroquercetin (Fig. 8) is characterized by a peak at 3290 cm^{-1}, which means a symmetrical and asymmetric stretch vibration of OH due to a multitude of intermolecular hydrogen bonds, while another bond at 2925 cm^{-1} represents CH stretch vibrations, the absorption band at 1617 cm^{-1} appeared due to the bending of HOH (Zhang *et al.*, 2017).

The presence of peaks in the infrared spectra characteristic of separate components of hydrogel is a confirmation of the presence of these substances in the composition of the obtained composites.

A significant criterion for the use of these patches as wound-healing dressing is the duration of release of the active substance from biocomposites. So, the curves of the release of the active substance from the studied plasters were plotted at the next stage.

The results showed that composite materials presented as patches with BC/chitosan/sodium fusidate hydrogel and BC/sodium alginate with $CaCl_2$/sodium fusidate have a longer antibacterial activity (up to 4 hours). At the same time, patches with BC/chitosan and BC / sodium alginate /SF hydrogel lose their activity in 1 hour (Fig. 9). This may be attributed to the interaction through chemical bonds of PH and chitosan and the crosslinking of sodium alginate with a solution of calcium chloride, which is much stronger than the adsorption forces binding the remaining components.

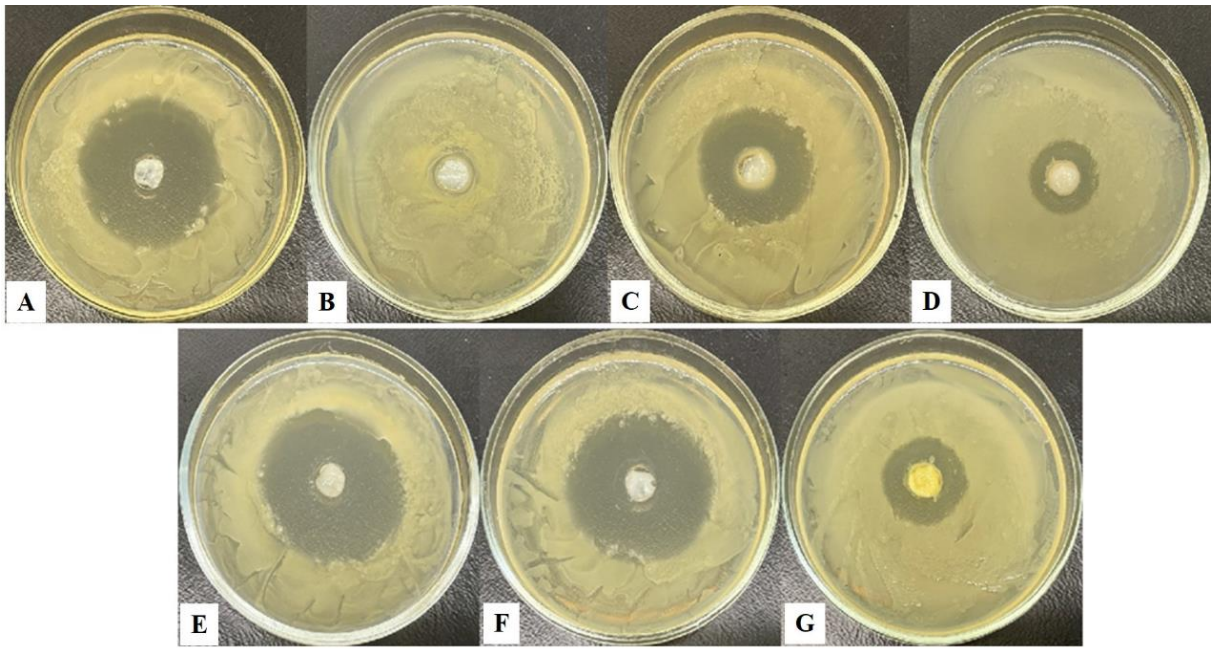

Fig. 5. Analysis of the antibacterial properties of the obtained hydrogels in respect to *S. aureus*: A – BC/SF; B – BC / chitosan; C – BC / chitosan / SF; D – BC / resveratrol; E – BC / sodium alginate / SF; F – BC/sodium alginate with $CaCl_2$/SF; G – BC/dihydroquercetin

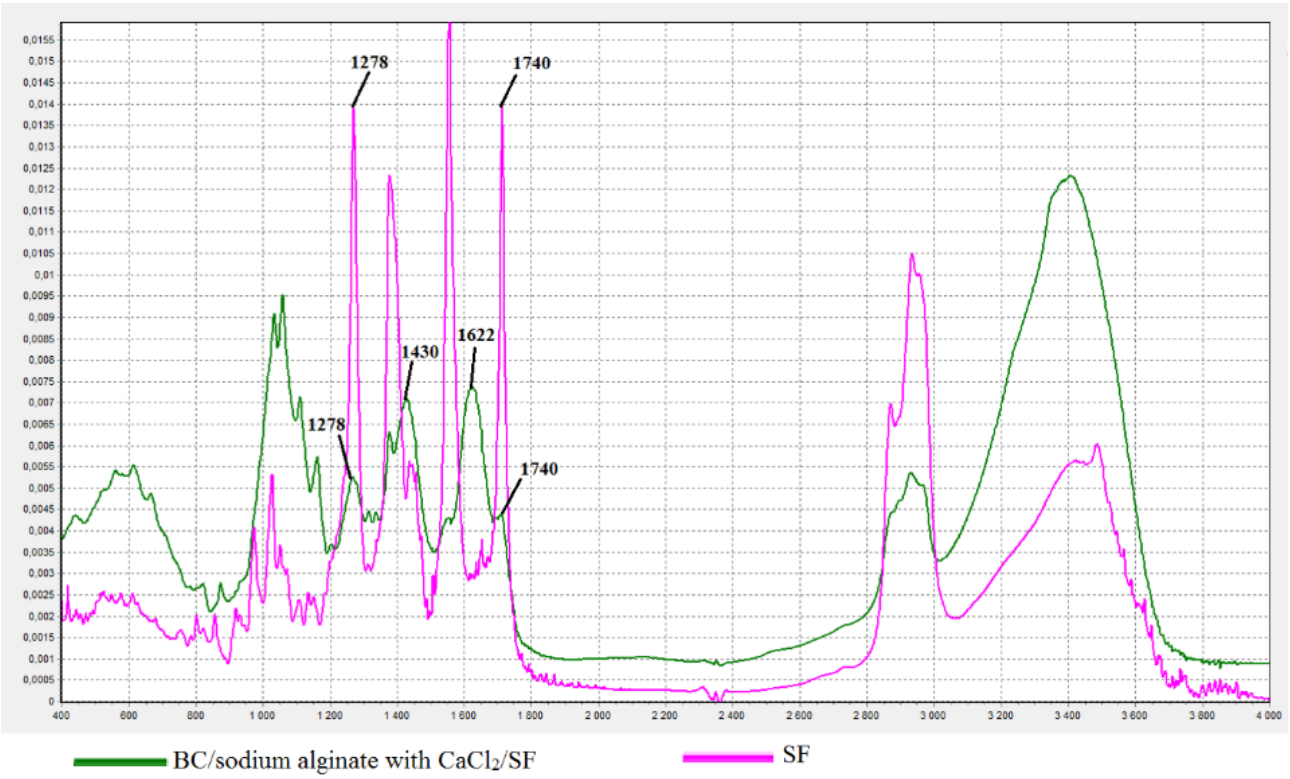

Fig. 6. Fourier-transform IR (FTIR) spectrum of BC / sodium alginate with $CaCl_2$ / SF and SF hydrogel

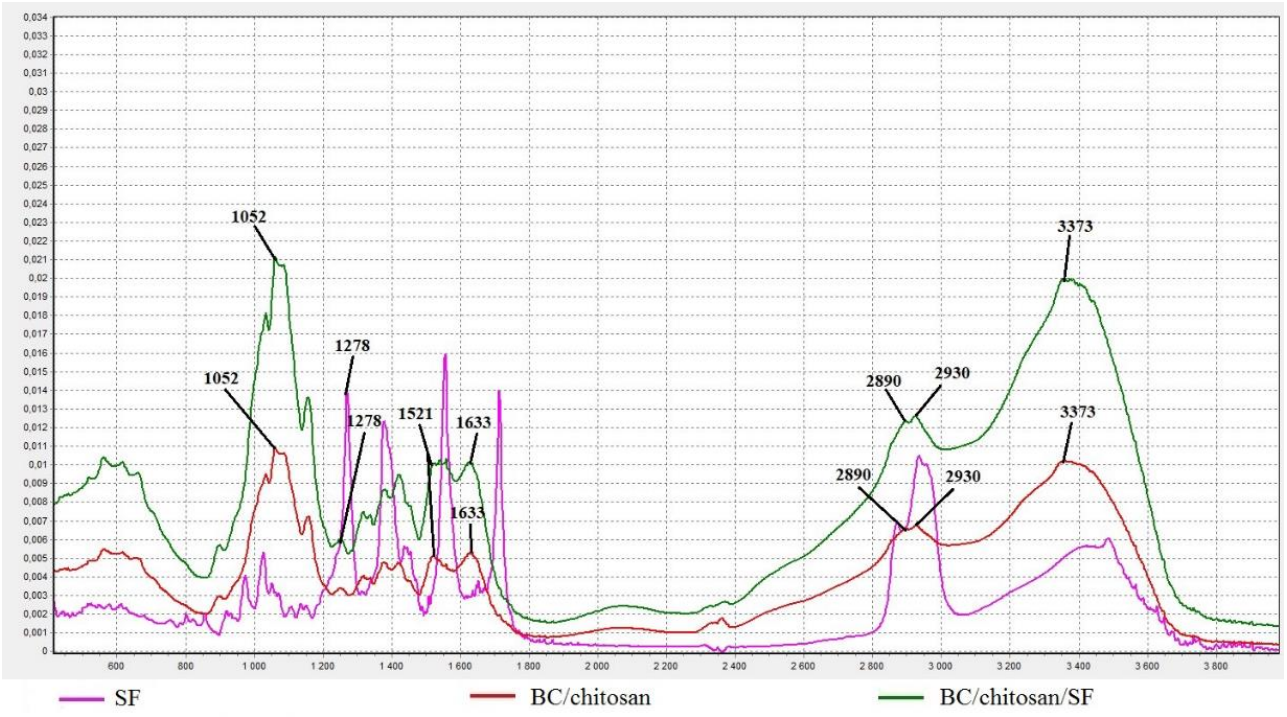

Fig. 7. FTIR spectra of BC/chitosan, BC/chitosan/SF and SF hydrogels

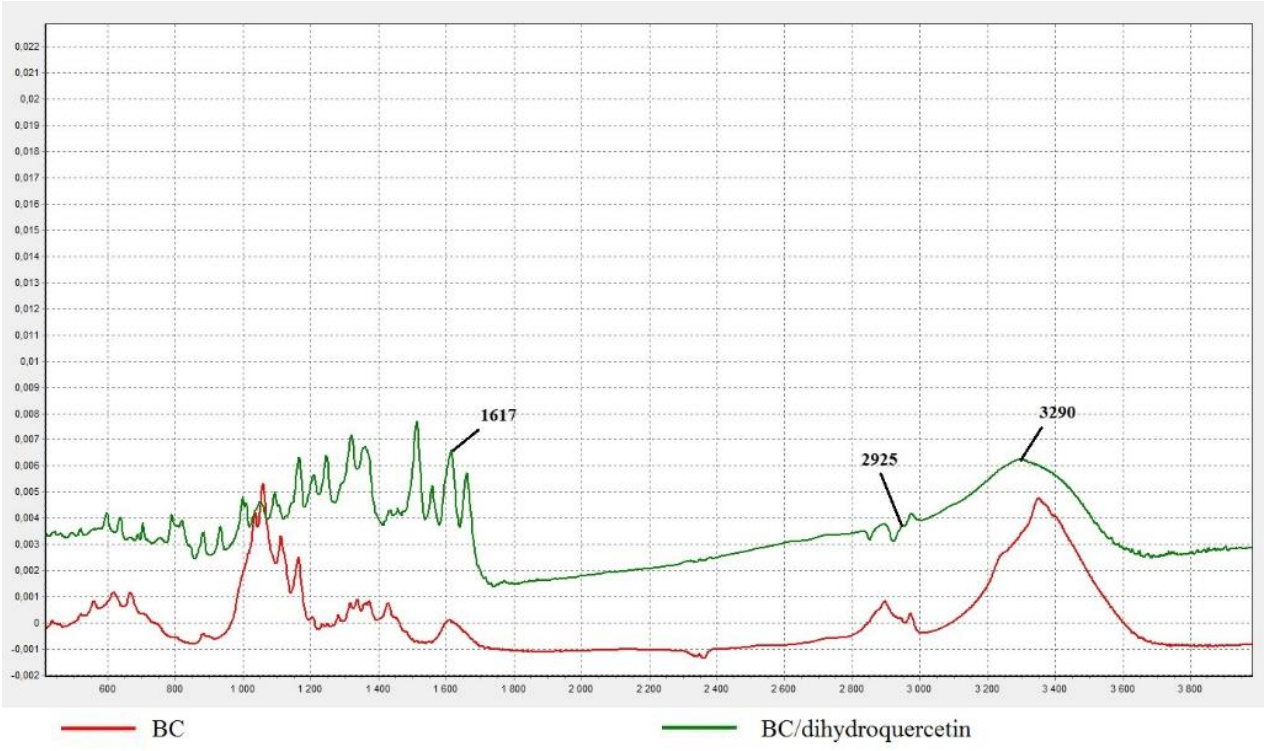

Fig. 8. FTIR spectra of BC and BC/ dihydroquercetin

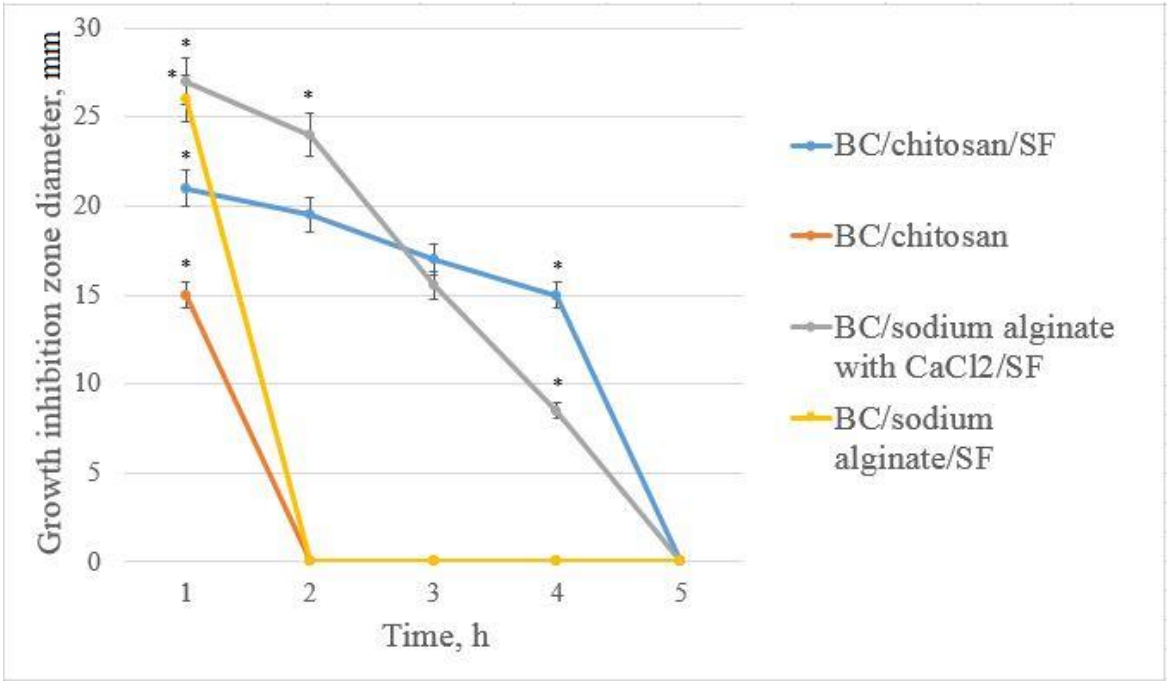

Fig. 9. Curves of the release of the active substance from patches

Changes in the content and ratio of lipid components, as well as changes in the activity and expression levels of enzymes associated with their metabolism, can affect the barrier function of the skin and its recovery after burn injury (Itaya & Tokudome, 2016).

At the first stage we analysed the change in the lipid composition of the skin after burn injury and after the use of biocomposites based on bacterial cellulose. The experiment showed that healthy skin contains the following phospholipid fractions: sphingomyelin (SM), phosphatidylcholine (PCH), phosphatidylserine (PHS), lysophosphatidylcholine (LPCH), phosphatidylethanolamine (PEA). In 30 minutes after the burn injury, the content of PCH remains almost the same, however, 7 days after the injury, following the use of BC/ dihydroquercetin, BC/sodium alginate with $CaCl_2$/SF and BC/chitosan/SF, its content increases significantly by 1.8, 4.2 and 3.7 times, respectively, compared with the injury time. With extension of the experiment time to 14 days, a significant decrease in the level of PCH compared to 7 days of observation was noted in a series of experiments with BC /sodium alginate with $CaCl_2$ /SF; BC/chitosan and BC/chitosan/SF by 49.1%; 31.5% and 36.8%, respectively. In 21 days after the injury, there is a 2.2-time increase in the level of PCH in the BC/chitosan variant of the experiment against the value at the time of injury. In other variants of the experiment, no significant changes are detected compared with the 14^{th} day of the experiment (Fig. 10).

The experiment showed that the level of PHS decreases by 24.7% against the level at the time of the injury. The use of BC/dihydroquercetin, BC/sodium alginate with $CaCl_2$/SF and BC/chitosan/SF causes the uprise in the level of SF by the 7^{th} day of observation by 1.8, 3.2 and 2.6 times, respectively, compared with the level at the time of burn injury. In the BC/chitosan variant of the experiment, the PHS content decreases by 54.9% against the control value. By the 14^{th} day of observation, the most distinct changes were noted in a series of experiments with BC/sodium alginate with $CaCl_2$/SF: the level of PHS increases 2.3 times compared to the injury time, exceeding the control value by 1.7 times. With the extension of experiment time to 21 days, the PHS level increases following the use of BC/dihydroquercetin, BC/sodium alginate with $CaCl_2$/SF and BC/chitosan against the level at the time of injury, reaching its level

on the 7th day of the experiment. At the same time, the content of PHS following the use of BC / chitosan also rises by 2.5 times compared to the level at the time of burn injury (Fig. 10).

In 30 minutes after the burn injury, the amount of sphingomyelin has also changed significantly. Thus, the SM content falls by 79.9% compared with its level at the time of injury. In 7 days after the injury, the most distinct change in the content is noted in the variants of the experiment with BC/sodium alginate with $CaCl_2$/SF and it increases by 7.2 times compared with its level after burn injury (Fig. 11). With extension of the damaging effect up to 14 days, the concentration of SM continues to increase following the use of BC/dihydroquercetin, BC/sodium alginate with $CaCl_2$/SF and BC / chitosan / SF and it exceeds its level in the variant of the experiment at the time of injury by 5.9, 5.3 and 5.7 times, respectively. On the 21st day of the experiment, the SM content falls in all variants of the experiment with BC-based biocomposite materials compared with the 7th day of the observation. Nevertheless, in a series of experiments with BC/ dihydroquercetin, BC/sodium alginate with $CaCl_2$ /SF, its level exceeds that one in the variant of the experiment at the time of injury by 2.9 times, remaining 40.9% lower than the control value (Fig. 11).

In the course of analysis, it was shown that the content of PEA is reduced by 94.0% compared with the values at the time of injury. In 7 days after the injury, a significant increase in its level is noted in all variants of the experiment with biocomposite materials. At the same time, by the 14th day of observation, the most pronounced increase in the content of FEA occurs in a series of experiments with BC / chitosan / SF and exceeds the variant of the experiment with injury by 41.3 times. The extension of the experiment to 21 days is accompanied by a change in the level of PEA compared with 14 days of observation in the experimental variants with BC / dihydroquercetin, BC / sodium alginate with $CaCl_2$ / SF and BC / chitosan / SF, almost reaching the control values (Fig. 11).

It is known that lysophospholipids formed during the activation of phospholipase A_2 are the actors of most pathological processes. In the course of study, it was found that after the lesion of the skin area, the amount of lysophosphatidylcholine (LPHC) increases and makes 29.1 micrograms / mg of lipids. The analysis of the LPHC level showed a significant increase in its level by the 7th day of the experiment by 3.2 times in the BC / dihydroquercetin variant of the experiment. Following the use of BC /sodium alginate with $CaCl_2$ /SF and BC/chitosan/SF, the content of LPHC increases by 6.6 and 6.5 times, respectively, compared with the level at the time of injury. In 14 days after the injury, the level of LPHC decreases in all variants of the experiment, but still significantly exceeds the control values. By the 21st day of observation, LPHC is not found during the use of BC/dihydroquercetin, BC/sodium alginate with $CaCl_2$/SF and BC/chitosan. In the variant of the experiment with BC/chitosan/SF, the level of LPHC exceeds its value after burn injury by 2.8 times (Fig. 11).

The intensification of lipid peroxidation (LPO) leads to various disorders at the level of separate enzyme systems and the whole cell. It has been established that when the functional state of the cell changes, a redistribution of fatty acids of excitable formations occurs. Moreover, the effector role of polyunsaturated fatty acids and their derivatives were revealed (Sawada *et al.*, 2021). Therefore, it was of interest to study the quantitative distribution of fatty acids that make up the lipid phase of the skin after a burn and the use of BC-based biocomposites. The fatty acids of the skin's lipid fraction normally contain of 17 fatty acids, such as: C12:0 (lauric), C13:0 (tridecyl), C14:0 (myristic), C14:1 (myristoleic), C15:0 (pentadecanoic), C16:0 (palmitic), C16:1 (palmitoleic), C17:0 (margarine), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), C18:3 (linolenic), C20:0 (arachinic), C20:2 (eicosadiene), C22:0 (begenic), C24:0 (lignocerine), C24:1 (nervonic) (Fig. 12).

The number of saturated FAs in a healthy skin comprises 51.7%, and unsaturated - 48.3%. The saturation coefficient is 0.5. After causing a II-IIIrd degree burn, 30 minutes later, chromatogram showed that the following FAs were not present in the control sample: C20:3

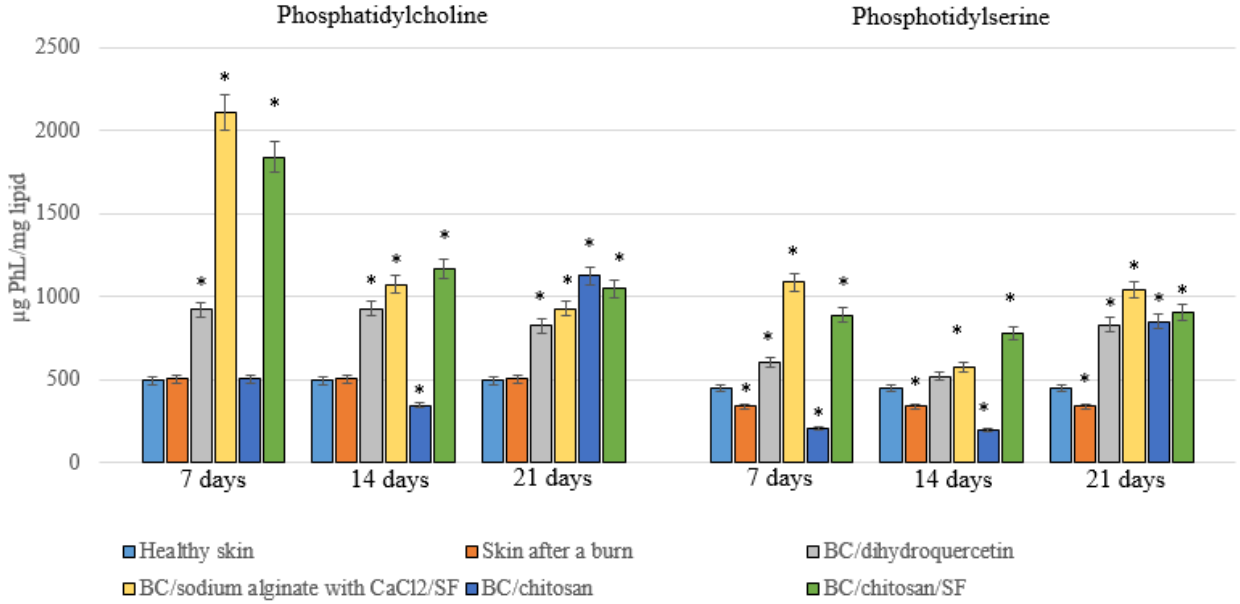

Fig. 10. Changes in phospholipids content in a rat's skin after burn injury (* – $P \leq 0.05$)

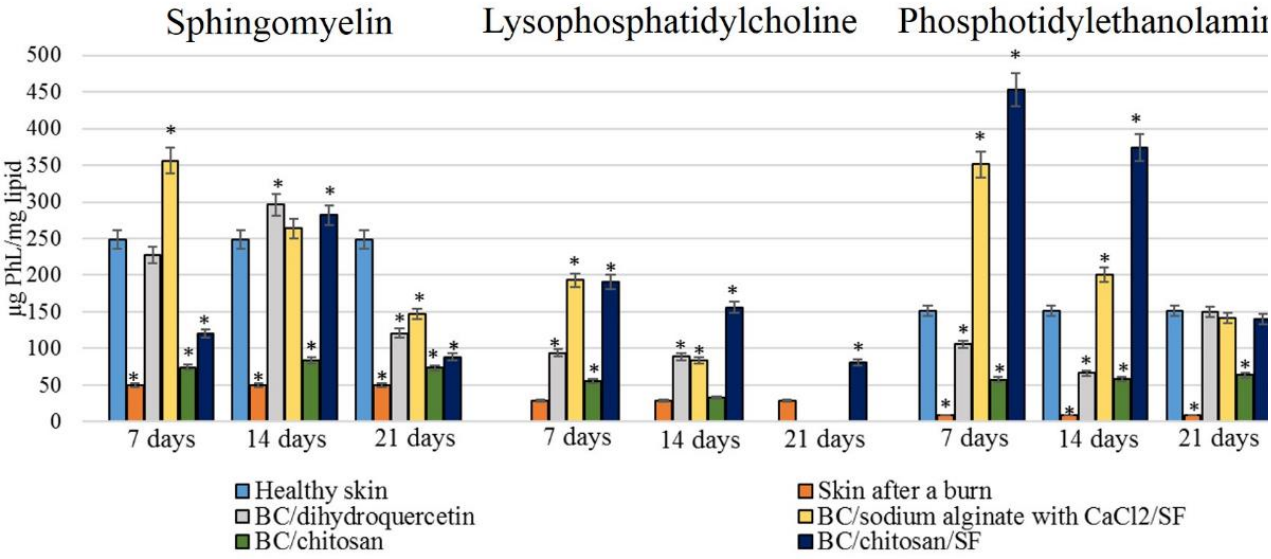

Fig. 11. Changes in phospholipids content in a rat's skin after burn injury (* – $P \leq 0.05$)

(dihomo-u-linolenic), C21:0 (geneicocylic), C22:1 (erucic). Moreover, there was a sharp increase in pentadecanoic, palmitic and eicosadienoic acids on average by 4, 12 and 8 times respectively. At the same time, the saturation coefficient decreased and averaged 0.4. In 7 days after the injury and application of BC/dihydroquercetin covering the wound, the number of all saturated FAs decreased. Out of unsaturated FAs, there was a decrease of C18:1, C18:3, C20:2 on average by 2 times, as well as 24:1 by an average of 1.5 times against the control values.

It was shown that in the variant of the experiment with BC/sodium alginate with $CaCl_2$ /SF, in 7 days after the injury C14:1, C20:0 and C24:1 were not detected. At the same time, C15:0, C18:2 and C18:3 dropped to the control values, and C18:0, C16:1 and C20:3 rose on average by 50.0; 4.0 and 10.0 times, respectively,

against the control values. The use of BC/chitosan in the same series of experiments was accompanied by a decrease of saturated FAs, namely: C13:0 and C14:0 by an average of 8.0 times; C15:0 by an average of 10.0 times; C18:0 by an average of 3.0 times and C20:0 by an average of 6.0 times against the control values. The number of unsaturated FAs also dropped, namely: C16:1 by an average of 4.0 times; C18:1 by an average of 15 times; C20:2 by an average of 2.0 times; C24:1 by an average of 3.0 times compared to the control values. The saturation coefficient in this variant of the experiment was lower against the control value by 40% and is 0.3. In 7 days after applying BC /chitosan/SF hydrogel to the damage area no traces of C12:0, C20:2, C20:3, C20:4, C22:0, C22:1, C24:1 were detected. The number of saturated FAs C15:0 and C20:0 decreased on average by 16.0 and 1.5 times respectively, compared with the control values. The number of unsaturated FAs C18:1, C18:2 and C20:2 decreased by 15.0, 4.0 and 2.0 times respectively, against the control values (Fig. 12). At the same time, the saturation coefficient amounts to 0.6, which is 20% higher than the control values.

In 14 days after the injury, the most obvious effect on the fatty acid composition of the lipid fraction of the skin was produced by BC / sodium alginate with $CaCl_2$ / SF. In this version of the experiment, the amount of C16:0 rose by an average of 2.0 times and C18:0 by an average of 40.0 times, as well as the level of C24:0 dropped by an average of 1.5 times. In respect to unsaturated fatty acids, one should draw attention to the decrease in the concentration of C16:1 by an average of 5.0 times; C18:1, C18:3 by an average of 2.0 times and the increase in the amount of C18:2; C20:2 and C24:1 by an average of 2.0; 9.0 and 2.5 times, respectively, compared with the control values. It was shown that in this version of the experiment the lipid phase saturation coefficient becomes normal, and the value reaches the control level of 0.5 (Fig. 13).

By the 21st day of observation, the least distinct changes in the fatty acid composition of the skin compared to the control values were marked following the use of BC/sodium alginate with $CaCl_2$ / SF after the burn injury. In this version of the experiment, we have the increase of C18:2; C18:3; C22:1 and C24:1 on average by 2.0; 15.0; 2.1 and 2.0 times, respectively, compared with the control values. In a series of experiments with BC /chitosan/SF, there was a significant redistribution in the quantitative content of most fatty acids against the control values: C20:0 and C22:0 decreased by 5.0 and 10.0 times respectively, against the control values. The concentration of C15:0, C16:0 and C18:0 rose on average by 2.0; 2.1 and 20.0 times respectively. The level of unsaturated FAs C18:1 and C24:1 decreased by an average of 2.0 times against the against the control values. At the same time, C18:2, C18:3 and C20:2 rose by 2.5, 3.0 and 16.0 times respectively, against control values (Fig. 14).

Since LPO products are damaging agents for cell membranes and intracellular organelles, at the next stage we made the analysis of the change in the content of LPO products – diene conjugates (DC) and dienketones after burn injury and the use of biocomposite materials based on bacterial cellulose hydrogel and biocompatible polysaccharides, such as chitosan and alginate, fusidic acid and dihydroquercetin.

Upon the completion of the analysis, it was revealed that after a II–III degree burn, the number of diene conjugates and dienketones increased by 2.0 and 10.0 times, respectively, compared with the control values. In 7 days after the burn injury, in the variant of the experiment with BC/chitosan, the most obvious decrease in the level of DC was observed by an average of 2.8 times compared with the time of burn injury. At the same time, the maximum drop in the content of dienketones by 2.7 times compared with the time of injury in the same series of experiments was noted after the use of BC /chitosan /SF (Fig. 15).

The extension of the experiment time to 21 days was accompanied by a similar dynamic: the use of BC / sodium alginate with $CaCl_2$/SF and BC/chitosan facilitated the decrease in DC by an average of 3.4 times, and the level of dienketones decreased by 5.5 times compared to the burn in the BC/chitosan experiment variant (Fig. 16, 17).

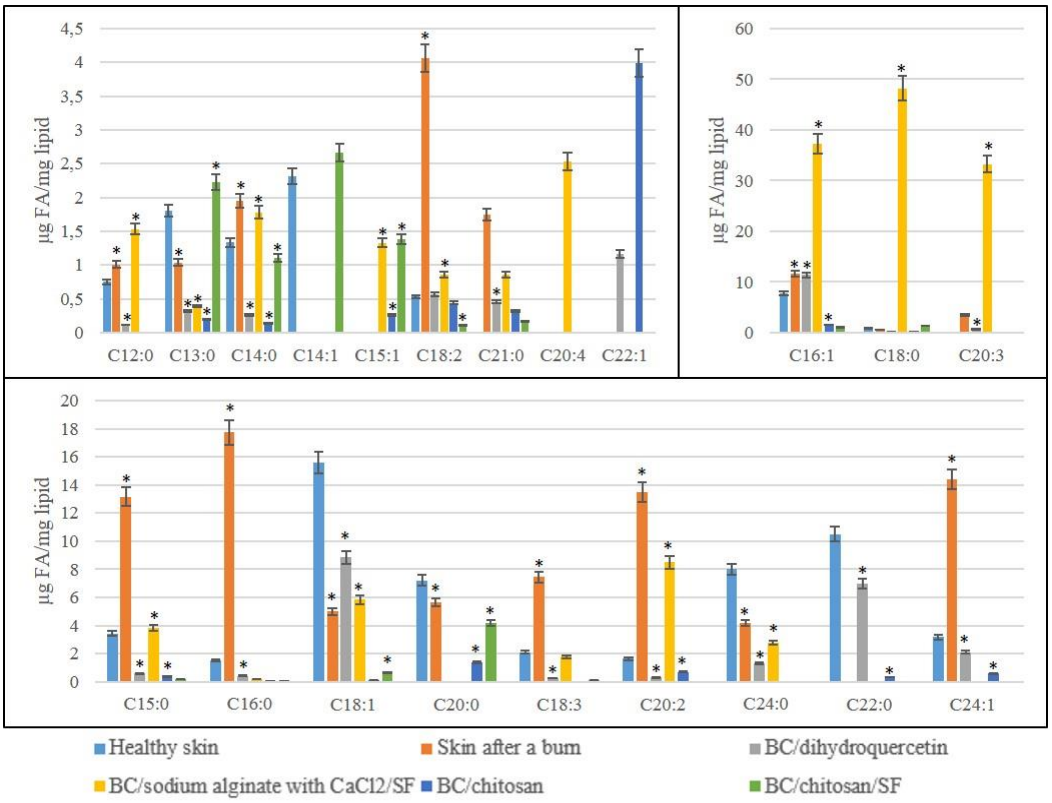

Fig. 12. Changes in the fatty acid composition of rat's skin phospholipids on the 7th day after burn injury (* – $P \leq 0.05$)

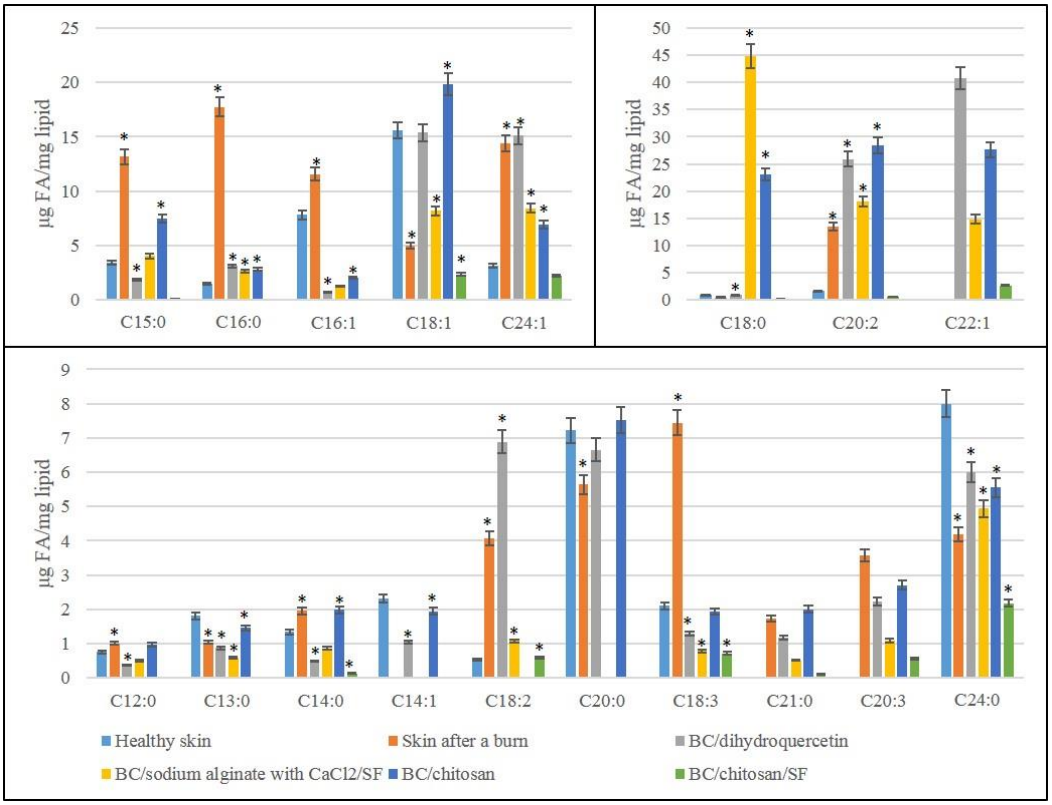

Fig. 13. Changes in the FAs composition of rat's skin phospholipids on the 14th day after burn injury (* – $P \leq 0.05$)

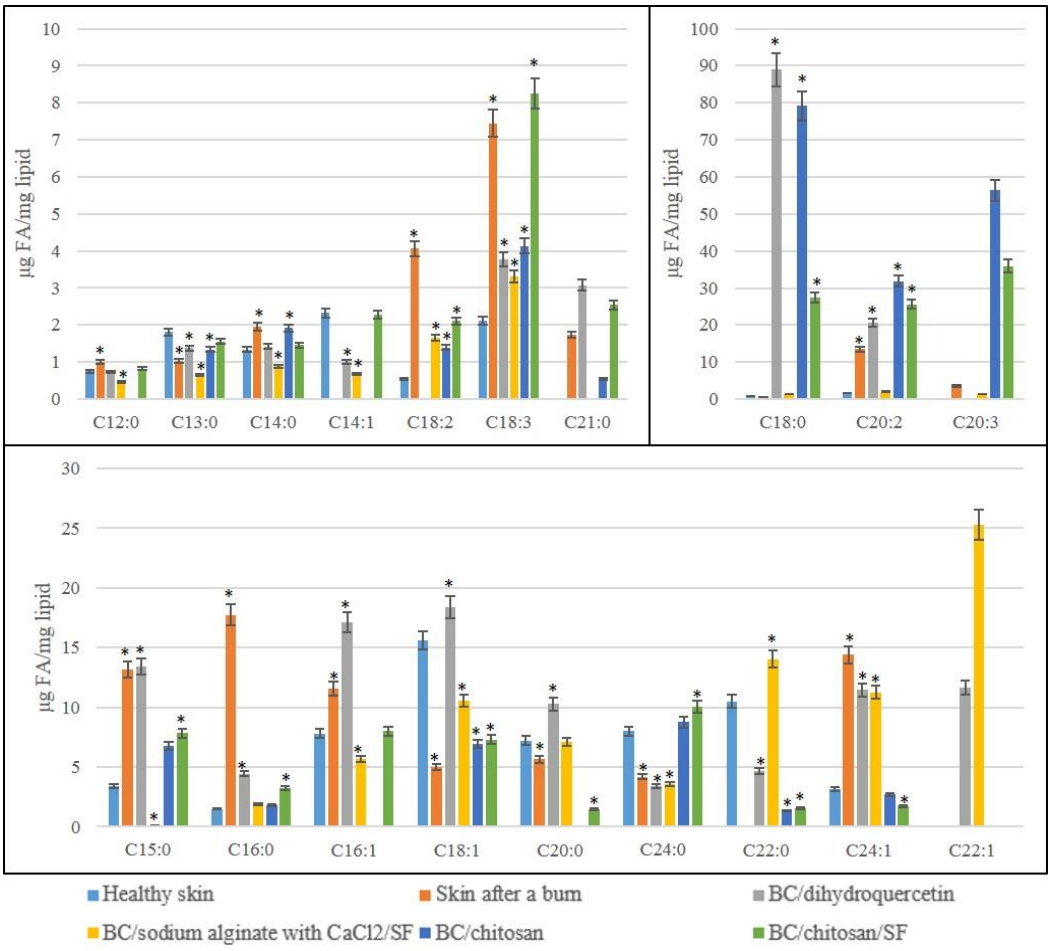

Fig. 14. Changes in the FAs composition of a rat's skin phospholipids on the 21st day after burn injury (* – $P \leq 0.05$)

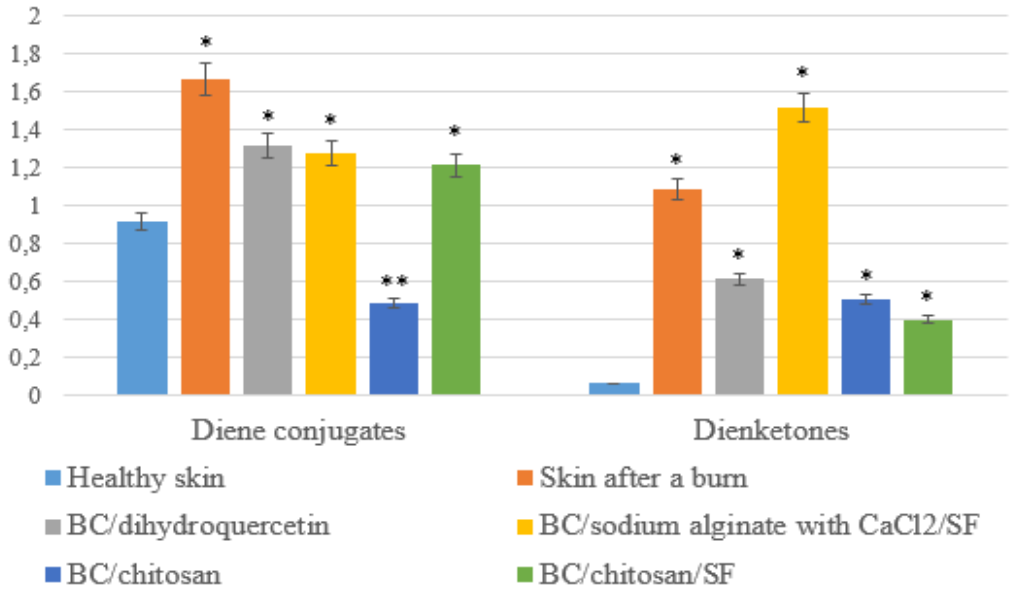

Fig. 15. The content of the LPO products 7 days after burn injury (* – $P \leq 0.05$)

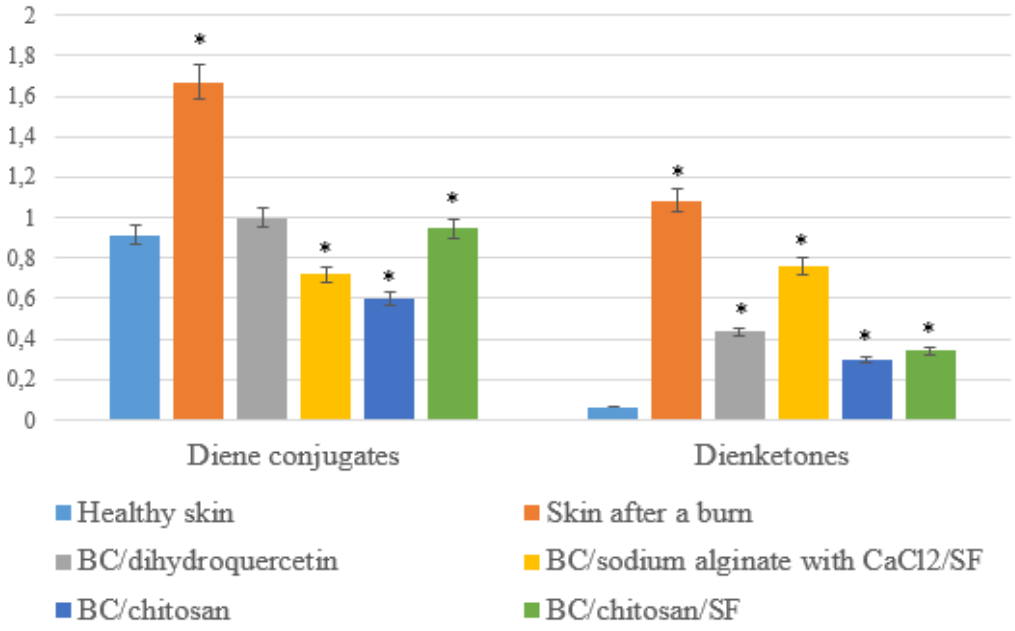

Fig. 16. The content of the LPO products 14 days after burn injury (* – $P \leq 0.05$)

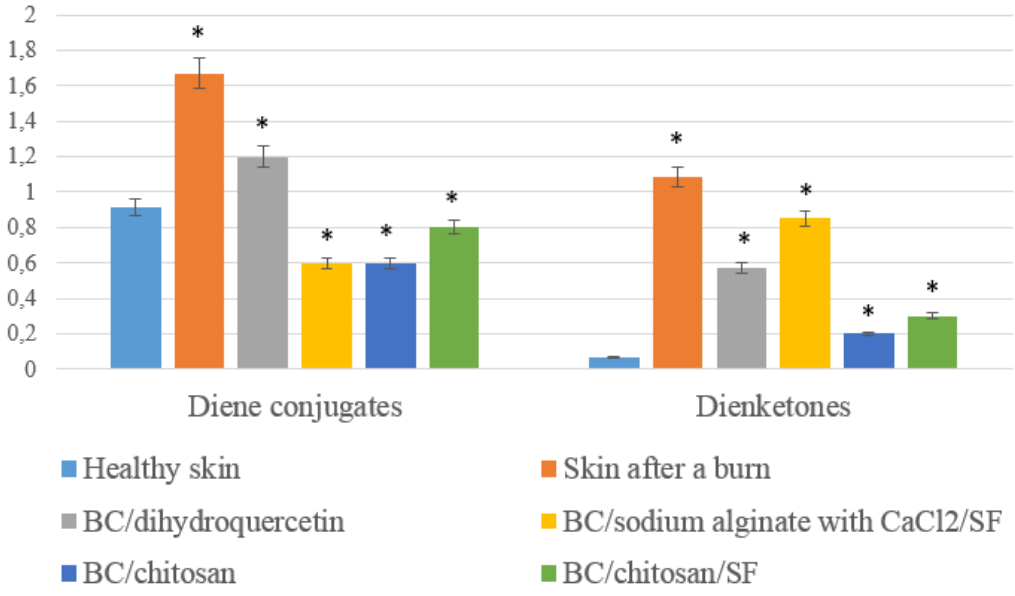

Fig. 17. The content of the LPO products 21 days after burn injury (* – $P \leq 0.05$)

The findings obtained are consistent with those of reviewed literature and point to the increase in the formation of reactive oxygen species and the intensification of LPO processes in burn injury (Kausar *et al.*, 2021). Therefore, the use of biocomposite materials BC/chitosan/SF, BC/chitosan and BC/sodium alginate with $CaCl_2$/ SF facilitated a significant drop in the level of LPO products already in the initial stages after burn injury. The use of these biomaterials limits the activation of lipid peroxidation, preventing reactions of both enzymatic and non-enzymatic peroxidation, which is one of the main factors for the effective course of wound healing after burn injury.

Discussion

Hydrogel is three-dimensional meshwork where hydrophilic polymers are crosslinked with each other and can swell, absorbing a large amount of water or biological fluids maintaining the structure of their meshwork. These compounds are very similar to living tissues owing to their high moisture capacity, permeability and consistency. Recently, there has been a great deal of research efforts pertaining to the preparation of transdermal membranes from polysaccharides. Among the most widely used hydrophilic polymers in the synthesis of hydrogel, polysaccharides have had several advantages over synthetic polymers (Abasalizadeh *et al.,* 2020). These polysaccharides are biocompatible, biodegradable, non-toxic, and have important medical properties, such as antimicrobial and hemostatic. Owing to these properties, hydrogel made from above polymers may be indispensable as wound dressing.

In addition, in order to ensure a constant influx of biologically active substances and the effectiveness of the use of BC-based wound coverings, it is possible to include various substances in its composition, such as biocompatible polysaccharides, antibiotics and antioxidants. In particular, fusidic acid is an antibiotic synthesised from the fungus *Fusidium coccineum*, which belongs to the class of steroids, but voids a corticosteroid effect (Liyaskina *et al.,* 2017). Currently, the acid is available in many medications administered orally (pills and suspensions), intravenously and locally (cream and ointment). It was shown that intensive release of the antibiotic in the first hours is necessary to prevent the spread of infection in the initial stages after the burn, while constant release of the active substance during the following hours is required to maintain antimicrobial activity (Zmejkoski *et al.,* 2018). As a results, for a more effective manifestation of the wound-healing properties of bacterial cellulose-based hydrogel, chitosan should be used in combination with SF and sodium alginate is cross-linked by a calcium chloride solution.

It is known that the skin consists of epidermal and dermal layers protecting the body from physical, chemical and microbial damage. The corneous layer is the outer layer of the epidermis, and it consists of keratinocytes and intercellular lipids. The diversity of the individual composition of phospholipids, the high rate of their metabolism, the presence of fatty acids in their composition, shaping the physical state of the bilayer and the ability to oxidize, indicates their active participation in the normal functioning of the skin. Moreover, the quality of the fatty acids (FAs) composition of skin lipids is one of the most important indicators of its functional state. Fatty acids are involved in the formation of the membrane's hydrophobic zone and the determination of its phase state. The existence of unsaturated fatty acids in the phospholipids of membranes causes their susceptibility to various influences, in particular peroxidation. Therefore, because of burn injury to the skin, we have observed the decrease in the content of sphingomyelin, phosphatidylserine, and phosphatidylethanolamine, as well as the accumulation of lysophosphatidylcholine and lipid peroxidation products. It was revealed that burn injury leads to a significant redistribution in the fatty acid composition of the skin, which is accompanied by the decrease in the saturation coefficient characterising the microviscosity of the lipid bilayer.

It is known that the processes of free radical oxidation are observed in all lipoprotein structures and membranes, which is a normal physiological process. When the cell membrane is damaged, an imbalance between antioxidants and pro-oxidants occurs towards activation of lipid peroxidation (LPO) (Sredoja Tisma *et al.,* 2021). The change in the quantitative content and fatty acid composition of separate lipid fractions is explained by the intensification of lipid peroxidation, as the presence of unsaturated fatty acids in phospholipids makes them most susceptible to oxidation.

Upon comparative analysis of the effect of various biocomposite materials on regeneration processes in the skin's lipid phase, we were able to show that hydrogel based on BC/chitosan/SF and BC/sodium alginate with $CaCl_2$/SF produce the most obvious effect on stabilisation of the

content of membrane phospholipids, restoring the FAs composition of the skin and reducing the LPO, which is explained by the constant influx of active substance from hydrogel composites, and the crosslinking of sodium alginate with calcium chloride provides a longer release of sodium fusidate from biocomposite materials, shortening the recovery time of the skin after burns.

Data availability: the data used to support the findings of this study are included within the article and the original data used to support the findings of this study are available from the corresponding author upon request (Marina V. Parchaykina; e-mail – mary.isakina@yandex.ru).

Conflict of interest: the authors declare that there is no conflict of interests regarding the publication of this paper.

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