PATHOGENETIC ASPECTS OF REGENERATION IN EXPERIMENTAL PURULENT WOUNDS AND METHODS FOR THEIR OPTIMIZATION

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Abstract. *The problem of treating complications of purulent wounds has remained relevant for many years. Despite the introduction of new and improvement of existing treatment methods in recent years, the incidence of purulent soft tissue lesions remains high. Our research focused on the concept of local treatment of purulent wounds of the skin and soft tissues using new multicomponent medications. This concept is aimed at implementing an individual approach in the treatment of experimental rats with purulent processes of the skin and soft tissues.*

Purpose of the study: *to pathogenetically substantiate the effectiveness of gel-like collagen with phylllabin on the healing of purulent wounds during their complex treatment under experimental conditions.*

Research materials and methods: *Experimental studies were conducted on 170 non-breed white male rats weighing 190-240 g, kept in the TSMU vivarium. "Experimental Modeling of Purulent Wounds" (Certificate No. 1394, 2023) was used to model purulent wounds of soft tissues in rats. A gel-like collagen coating material with fillalbin, obtained by biomedical engineering methods, was applied to the purulent wound (Scientific work was carried out by Seomashko N.Yu., Aropova S.F., Abidova A., Khegay L.N., Iriskulov B.U. and others on the basis of the patent No. IAP 06668 "A means with regenerative and antimicrobial properties for covering wounds of various origins," as well as the grant No. 211/14 of the Ministry of Innovative Development of the Republic of Uzbekistan). Its effectiveness was assessed in dynamics using bacteriological and enzyme immunoassay methods.*

Results: *In the main group of rats receiving collagen gel with fillalbin, due to the antiseptic effect of fillalbin, a decrease in the number of bacteria was observed on the 7th day, with their complete death by the 10th day. We noted that the response rate indicators in systemic inflammation syndrome caused by the purulent process in the wound, namely the levels of interleukins (IL-1β, IL-2, IL-10), reached normal values by the 10th day. A critical progression of procalcitonin levels was also observed between 24 and 36 hours, indicating the high effectiveness of collagen in relation to these changes. In addition, the influence of vascular-endotelial growth factor (VEGF) on the formation of connective tissue architectonics was studied in dynamics.*

Conclusion: *As a result of our comprehensive treatment with biomedical cell coating material, due to the improvement of pathophysiological indicators in dynamics on average 4 days before purulent wounds compared to the comparison group, by observing the complete healing of the wound, it was proven that it is medically effective for our use in purulent wounds of soft tissues.*

Keywords: *phyllalbumin, gel-like collagen, procalcitonin, interleukin, VEGF, biomedical engineering, coating material.*

Introduction. A literature analysis showed that the results of national studies from more than 195 countries worldwide show that the incidence of skin and subcutaneous tissue lesions of various etiologies is 8.24% [1]. At the same time, the incidence of purulent ulcer inflammation reaches 45% of the total number of skin ulcers in the surgical profile, leading to disability and mortality ranging from 25% to 50% [2].

In the Russian Federation, more than 12 million cases of purulent wounds are registered annually, and 18% of the population receive accidental or postoperative surgical infectious wounds annually [3]. The share of open injuries is 16.7%, while according to global statistics, it is 12.3% [4.5]. From the perspective of treatment choice and prognosis, open injuries of the limbs are considered one of the most severe types of injuries, accounting for between 21% and 30% of all injuries [6,7].

A similar situation is observed in the regions of the People's Republic of China. In these areas, up to 5 million patients with purulent soft tissue ulcers are registered annually [8,9].

In the USA and Western European countries, 10% of hospitalized patients are patients with surgical soft tissue infections. The annual economic damage from this is estimated at 9-10 billion US dollars [10,11]. It is well known that the most effective treatment method for patients with surgical soft tissue infections loses its effectiveness if it is not combined with technologies that facilitate purulent wound cleansing and create favorable conditions for recovery processes [12,13].

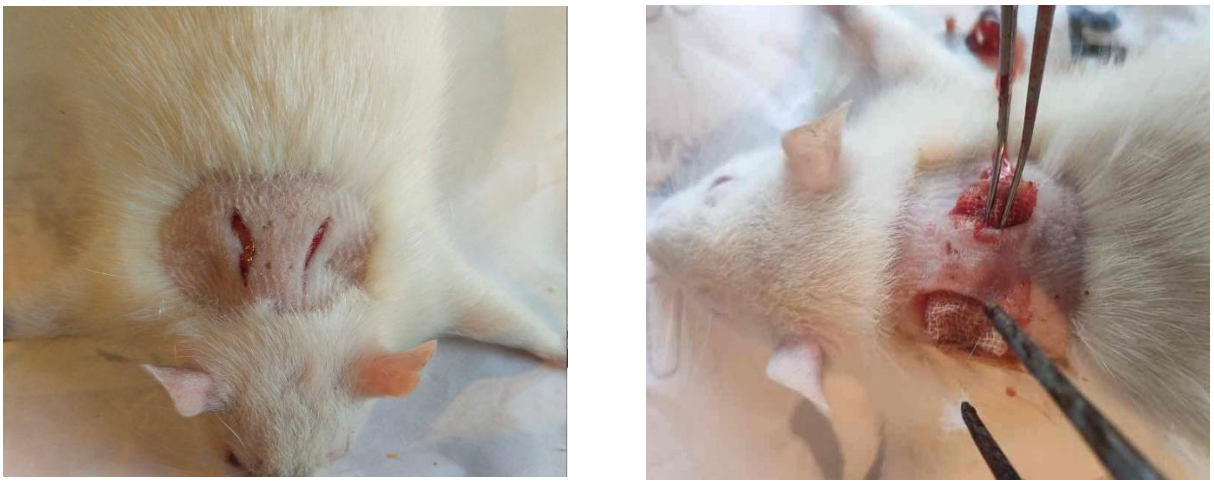
In the structure of nosocomial infections, the prevalence of surgical infections of the skin and soft tissues (post-operative, postoperative, and post-injection complications) reaches 36%. Approximately 2 million patients with purulent ulcers of the skin and soft tissues are treated annually in polyclinics, the majority of whom (more than 75 percent) are of working age. The duration of loss of working capacity in patients undergoing outpatient treatment is 13.6-17.6 days, and in patients undergoing inpatient treatment, it is 18.5-23.8 days. It was also established that the frequency of postoperative purulent-septic complications in traumatological hospitals ranges from 12% to 31% [14,15].

Materials and methods. Experimental studies were conducted on 170 sterile white male rats weighing 190–240 g kept in the TDTU vivarium. There were 10 intact rats. After surgical intervention, rats were divided into 3 groups according to treatment methods: control group – 64 rats were subjected to a purulent wound model on the soft tissues between the scapulae; comparison group – 54 rats were subjected to the aforementioned model, no fatality was observed, and the wound area after surgery was treated with a levomekol ointment; main group – 42 rats were subjected to a model, and gels of collagen with phyllalbin were applied once daily for 10 days. Research methods used enzyme-linked immunosorbent assays to detect IL-1β, IL-2, IL-10, procalcitonin, and histological methods.

Surgery. In our proposed method (Ergashev U.Yu., Abdusalomov B.A. "Method of experimental modeling of purulent wounds." Rationalization proposal. Certificate No. 1394) to create a model, a rat excrement was taken into a Petri dish before the manipulation, mixed with distilled water in a 1:4 ratio, passed through a 4-layer gauze, and an autocal suspension was prepared. During the study, after general anesthesia of the laboratory rat with ether, the experimental animal was placed on its stomach on A.I. Sechenov's table. In the upper part of the rat's scapula, a hair removal measuring 3.0 x 3.0 cm is performed. The sterilized area is treated with an antiseptic, and the skin and subcutaneous tissues are incised with a single-use scalpel along the longitudinal direction of the body at a distance of 1.0 cm from each other into 2 parallel incisions up to 2.0 cm long at a depth of up to the muscle fascia.

Subsequently, the skin and subcutaneous tissues are separated along the entire length of the incision using blunt and sharp methods.

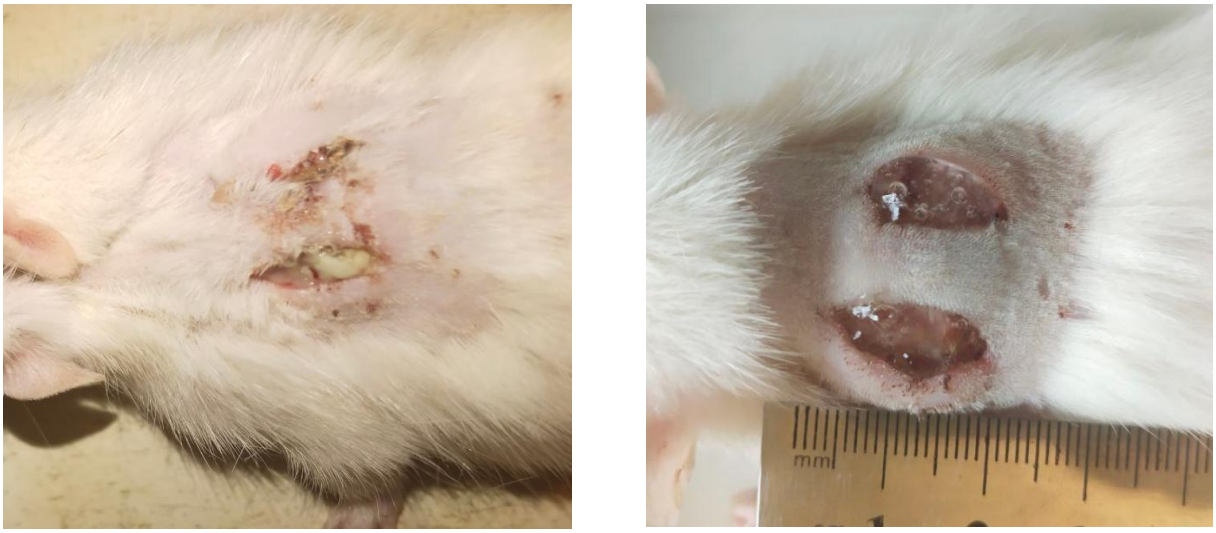
As a result, a unique "bridge" is formed. The facies under the bridge and the muscle are compressed using Kocher's forceps to form the affected area (see Fig. 1a).



a b

Figure 1. **Making a "bridge" incision between the scapulae (a), Placing a swab impregnated with an autocal suspension between the soft tissues (b)**

A sterile 4-layer napkin measuring 2.0x2.0 cm is dipped into the resulting suspension of autocalcal feces. A napkin impregnated with autocal suspension is passed under the resulting "bridge" (see Fig. 1b). Since the napkin is swollen, it does not slip under the "bridge" and there is no need for additional fastening. After 48–72 hours, the "bridge" skin dries up and forms scabies. The scabbard is cut within the boundaries of healthy tissues, and there is no need for anesthesia. After that, the napkin will fall off on its own. A purulent wound measuring 2.0 x 1.0 cm forms beneath it (see Fig. 2a).



a b

Figure 2. **Purulent process appearing after 3 days (a). Stage of surgical treatment of the wound and application of phyllalbine collagen (b)**

After 72 hours, the wound suppurates; the purulent wound is cleaned of pus using traditional antiseptic preparations once a day, and a local gel-like collagen preparation with phyllalbin is applied to the wound (see Fig. 2b). To measure the area of a purulent wound in experimental rats, we used Postnikov's millimeter paper (1949).

Special research methods. Currently, the reaction of systemic inflammatory syndrome plays an important role in wound healing compared to purulent-necrotic lesions of the body. These include cytokines produced by monocytes and tissue macrophages. Their quantities are involved in the continuation or healing of inflammation. Enzyme-linked immunosorbent assays were performed on a Rayto analyzer using the reagents Interleukin-1β, Interleukin-2, Interleukin-10, procalcitonin, and tissue growth factor VEGF-A from Vector Best (Russia).

Histological research methods. For morphological studies, the collected tissues (purulent wound, liver, and kidney) were fixed with 10% buffered formalin for 24 hours. The tissues were regularly processed on a STP120 carousel processor belonging to the German company Termo Fisher, after which the samples were placed in paraffin. Slices with a thickness of 3-4 μm were prepared on a NM 325 rotary microtome manufactured by the American company TermoFisher. To stain the sections with hematoxylin-eosin and Van Gieson, the serial sections underwent deparafination and dehydration.

Results. Results of cytokine studies. We also studied the activity of cytokines from blood plasma, which are responsible for wound inflammation and healing. After cytokines reach the inflammatory zone of monocytes and transform into tissue macrophages, cytokines are produced from their synthesis. These cytokines consist of a combination of pro- and anti-inflammatory cytokines (see Table 1).

In the control group, when checking the levels of IL-1β, IL-2, IL-10 on the 1st day, there was a decrease of 1.53 times ($p<0.001$) compared to the unchanged group; 1.38 times ($p<0.001$) decrease; An upward trend of 2.07 times ($p<0.001$) was observed. and on the 3rd day - 1.67 times ($p<0.001$); 1.32 times ($p<0.001$); We identified a trend toward a decrease of 1.52 times ($p<0.001$). On the 7th day, a 1.38-fold ($p<0.001$) increase in IL-1β was detected, indicating a high level of inflammation and the progression of the purulent process; IL-2 decreased by 1.40 times ($p<0.001$); We noted that IL-10 remained almost unchanged. A critical increase in IL-1β was identified on days 10 and 14, i.e., 1.41 times ($p<0.001$); 1.61 times ($p<0.001$) an upward trend was identified. IL-2 showed a downward trend, 1.47 times ($p<0.001$); 1.56 times ($p<0.001$). IL-10 increased to the highest critical point on days 10 and 14, 1.75 times ($p<0.001$), respectively, for IL-1β. By the 21st day, IL-1β and IL-10 reached normal values, while IL-2 remained at a high level of 0.353±0.00 pg/ml. IL-1β appears in blood plasma when pathogenic bacteria enter the body for the first time. During the illness periods, the level of this cytokine decreased, but during the recovery stage, it was even higher than normal, which allowed for the reduction to be considered a residual reaction of the immune system. IL-10 is an antagonist of anti-inflammatory cytokines and performs a regulatory function, reduces the activity of pro-inflammatory cytokines, and also performs key functions in wound healing. During the developmental stages of the disease, IL-1β increased proportionally, and during wound healing, it decreased to normal values.

Table 1.

Changes in the dynamics of systemic inflammatory syndrome response parameters

Day's	IL-1β, pg/ml	IL-2, pg/ml	IL-10, pg/ml
Intakt	0,122±0,004	0,344±0,011	0,122±0,004
Control group			
Day 1	0,08±0,003***	0,250±0,008***	0,59±0,019***
Day 3	0,073±0,002***	0,26±0,009***	0,08±0,003***
Day 7	0,168±0,005***	0,246±0,008***	0,127±0,004
Day 10	0,172±0,006***	0,234±0,007***	0,18±0,006***
Day 14	0,196±0,007***	0,221±0,006***	0,214±0,007***
Day 21	0,128±0,004	0,353±0,011	0,129±0,005

Comparison group			
Day 1	0,079±0,0026***	0,237±0,0079***	0,087±0,0029***^^^
Day 3	0,140±0,0044**^^^	0,298±0,0097**^	0,172±0,0056***^^^
Day 7	0,145±0,0048**^^	0,412±0,014**^^^	0,161±0,0050***^^^
Day 10	0,139±0,0042*^^^	0,371±0,012^^^	0,134±0,0044*^^^
Day 14	0,131±0,0040^^^	0,350±0,011^^^	0,128±0,0039^^^
Main group			
Day 1	0,071±0,0022***^&	0,253±0,0078***	0,080±0,0025***^^^&
Day 3	0,138±0,0045*^^^	0,372±0,013^^^&&&	0,164±0,0055***^^^
Day 7	0,142±0,0048**^^	0,364±0,012^^^&	0,155±0,0049***^^^
Day 10	0,123±0,0040^^^&	0,345±0,011^^^	0,123±0,0039^^^&

*Note: * - significant difference compared to the indicators of the intact group (* - p<0.05); ** - p<0.01; *** - p<0.001); ^ - significant difference compared to the control group (^ - p<0.05); ^^ - p<0.01; ^^^ - p<0.001); & - differences relative to the comparison group are significant (& - p<0.05; && - p<0.01; &&& - p<0.001)*

In the comparison group, the upward trend in IL-1β and IL-2 levels on the 1st day was slightly lower than in the control group. This is due to the antiseptic properties of the levomycol ointment, which blocks inflammation in the first days by acting on cytokine recognition receptors. At the same time, a downward trend was observed in IL-10 by 1.47 times (p<0.01). On the 3rd day, an increase in IL-1β by 1.92 times (p<0.001) was observed, which we explained by the fact that levomekol stimulates the production of cells responsible for the immune system. We found a trend toward an increase in IL-2 by 1.15 times (p<0.001) compared to the control group, while IL-10 levels also increased by 2.15 times (p<0.001) to date. By day 7, a trend toward a critical increase in all interleukins was observed compared to the control group, reaching 1.16 times (p<0.001); 1.68 times (p<0.001); 1.27 times (p<0.001). By day 10, due to the effectiveness of the treatment method, a decrease in all studied interleukins (except for IL-2) was observed compared to the control group, 1.24 times (p<0.001); 1.34 times (p<0.001); For IL-2, it increased by 1.59 times (p<0.001). On the 14th day, it proceeded with an equal trend compared to the unchanged group; only IL-2 remained at the same level, at 0.350±0.01 pg/ml. This was confirmed by the fact that they remained with high immunity to bacterial toxins and other infectious agents.

The cytokines in the blood plasma of rats in the main group remained virtually unchanged on the 1st day compared to the comparison group; a 1.02-fold (p<0.001) increase in IL-2 was observed, while no change was found in IL-10, which indicates the high immunomodulatory and antiseptic properties of the phyllalbin gel-like collagen. On the 3rd day, IL-1β was 1.31 times higher than in the comparison group (p<0.001); For IL-2, there was a trend toward an increase of 1.16 times (p<0.001). Since IL-10 was in the stage of wound healing, a decrease of 1.046 times was observed compared to the comparison group (p<0.001). By the 7th day, the inflammatory IL-1β reached its highest critical point, 0.142±0.00 pg/ml. A decrease in IL-2 and IL-10 was observed compared to the comparison group, amounting to 0.364±0.01 pg/ml and 0.155±0.00 pg/ml, respectively. The results obtained proved that phyllalbin gel-like collagen has a positive and desirable effect on immunomodulatory, antiseptic properties and wound healing activity compared to levomecol. We also determined procalcitonin levels, indicating the onset of inflammation in experimental rats with purulent wounds of the soft tissues between the scapulae.

Procalcitonin (PCT) is a propeptide of the hormone calcitonin, with a molecular weight of 12.795 kDa and 116 amino acid residues. The structure of PCT consists of 4 genes located on the short arm of chromosomes 11 and 12 in the genome, called the family of PCT genes. During translation, preprocalcitonin is synthesized, containing 141 amino acid residues with a molecular

weight of 15.466 kDa. In the endoplasmic reticulum of its cells, it releases 25 amino acid residues to form PCT, which is then broken down into the N-terminal fragment, calcitonin, and catacalcin, a peptide that regulates calcium levels, usually with the help of special enzymes.

When interpreting the procalcitonin (PCT) level in the blood plasma of experimental rats, we use the classification of Dandona P. et al., 1994 (Table 2).

Table 2.

Procalcitonin level, ng/ml	Possible state
>0,5	Local infection of the wound is possible; sepsis is excluded.
0,5-2	A local infection with a high probability of spread, developing sepsis.
2-10	Sepsis
10<	Sepsis severe, septic shock

Table 3.

Dynamics of changes in the procalcitonin PCT (ng/ml) level in the blood plasma of experimental animals, M±m

	Normative indicator	Control group	After treatment	
			Comparison group	Main group
Day 1	>0,5	1,5±0,046	1,4±0,049	1,2±0,045$^{***\wedge}$
Day 3		1,7±0,053$^{\&}$	1,5±0,053*	1,1±0,032$^{***\wedge\wedge\wedge}$
Day 7		1,8±0,065	1,6±0,062*	0,81±0,026$^{***\wedge\wedge\wedge\&\&\&}$
Day 10		1,5±0,053$^{\&\&}$	1,4±0,044$^{\&}$	0,62±0,021$^{***\wedge\wedge\wedge\&\&\&}$
Day 14		1,1±0,033$^{\&\&\&}$	0,98±0,037$^{*\&\&\&}$	0,53±0,018$^{***\wedge\wedge\wedge\&\&}$
Day 21		0,80±0,027$^{\&\&\&}$	0,68±0,022$^{**\&\&\&}$	0,38±0,013$^{***\wedge\wedge\wedge\&\&\&}$

*Note: * - significance of differences with the control group indicators (*-P<0.05; **-P<0.01; ***-P<0.001); ^ - significance of differences with comparison group indicators (^-P<0.05; ^^-P<0.01; ^^^-P<0.001). & - significance of the difference with the indicators of the previous days (& - P < 0.05; &&-P<0.01)*

It was established that PCT levels increase due to bacterial toxins after 1 day and reach levels observed in septic conditions after 3-5 days.

The PCT value in the blood plasma of the control group rats on the 1st day of the experiment was 1.5±0.31 ng/ml, which is 3.0 times higher (p<0.001) than in the unchanged group. On days 3-7, PCT increased to 1.7±0.41 ng/ml and 1.8±0.55 ng/ml, which was 3.4 and 3.6 times higher (p<0.001) than in the unchanged group, respectively. From the 14th to the 21st day of the experiment, a decrease in indicators was observed to 2.2 and 1.6 times, respectively (see Table 3).

The amount of PCT in the blood plasma of rats with purulent wounds in the comparison group on the 1st day of the experiment was 1.4±0.27 ng/ml, which is 1.07 times (p<0.001) lower than the values of the control group. On the 3rd day, PCT was 1.5±0.73 ng/ml, which was 1.3 times lower (p<0.001) than in the control group. On days 7–10, it was 1.12 and 1.07 times lower (p<0.001), respectively, than in the control group. On days 14–21, the PCT level decreased to 0.98±0.27 ng/ml and 0.68±0.37 ng/ml, which we attributed to the positive effect of the levomekol ointment applied to the wound defect in animals of the comparison group on the reparative processes in the wound.

It is known that PCT can manifest as an increase in its level in the blood even against the background of inflammation in the animal body. The increase in PCT levels in the control group rats was influenced not only by compensatory mechanisms but also by the inflammatory state of the wound surface against the background of purulent inflammation of the soft tissues.

The amount of PCT in the blood plasma of experimental animals with purulent wound defects in the main group was 1.17 ($p<0.001$) and 1.25 ($p<0.001$) times lower on the 1st day of the experiment compared to the comparison and control groups, and by the 3rd day by 1.36 ($p<0.001$) and 1.55 ($p<0.001$) times, respectively; On the 7th day, these indicators were 1.98 ($p<0.001$) and 2.22 ($p<0.001$) times lower, respectively. (see Fig. 3).

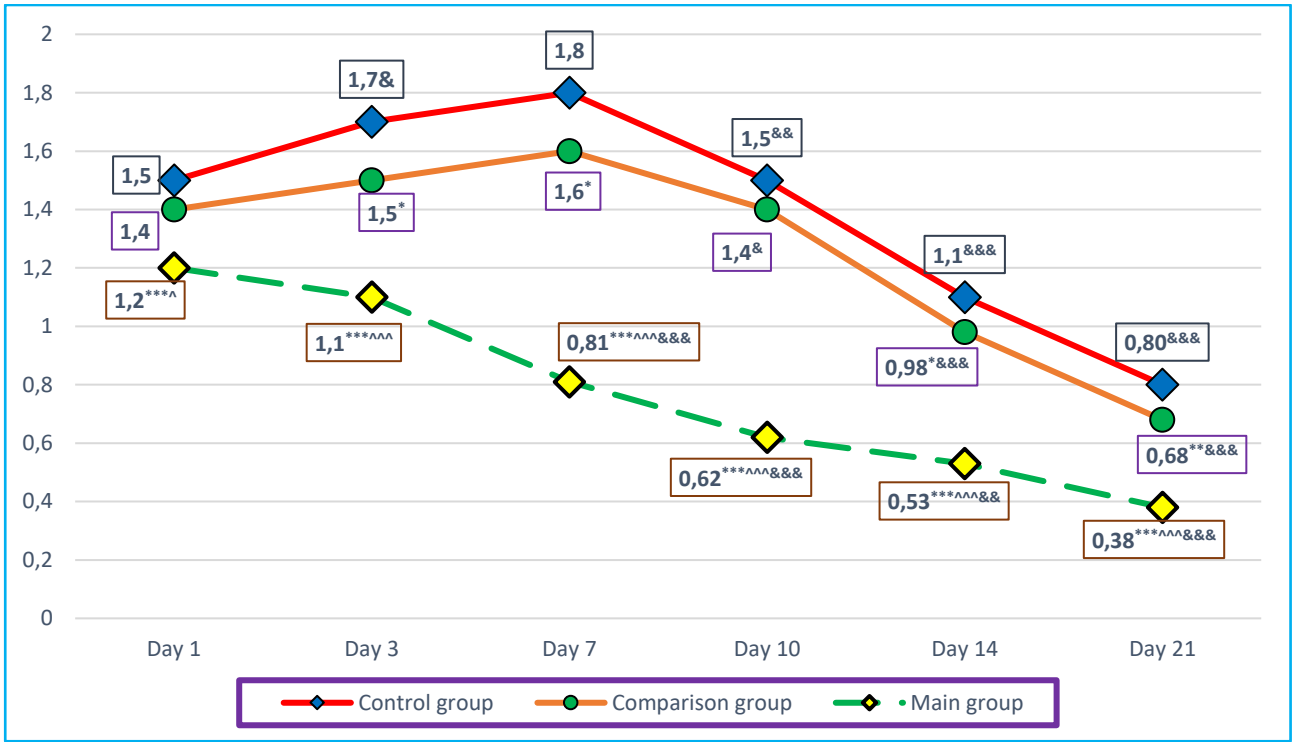


Figure 3. **Dynamics of changes in procalcitonin levels in the blood plasma of experimental rats**

*Note: * - significance of differences with the control group indicators (*-P<0.05; **-P<0.01; ***-P<0.001); ^ - significance of differences with comparison group indicators (^-P<0.05; ^^-P<0.01; ^^^-P<0.001). & - significance of the difference with the indicators of the previous days (& - P < 0.05; &&-P<0.01)*

The most potent stimulators of PCT secretion and release into the blood are bacterial bodies, endotoxins of gram-negative bacteria, exotoxins, and products of the life activities of gram-positive bacteria. At the same time, the stimulating role of gram-negative bacteria is approximately nine times higher than that of gram-positive microorganisms. Significant excretion of PCT occurs only during the body's systemic response to infection. Local inflammatory processes caused by bacterial flora do not lead to a significant release of PCT into the blood, but are caused by its toxins. The amount of PCT released during infection corresponds to the level of activity of the inflammatory response.

It has been established that increased PCT formation in sepsis occurs more frequently in parenchymal organs (primarily the liver) than in leukocytes. It was concluded that there are two activation pathways for PCT synthesis - the first is responsible for increasing blood calcium levels under normal conditions, while the second is not yet fully understood and resembles mechanisms specific to systemic inflammatory mediators. This pathway of activation can occur both directly—in

response to the effector cell's interaction with the bacterial antigen—and "retrograde"—in response to a systemic inflammatory response accompanied by a "mediator explosion." In short, PCT is a response to systemic inflammatory syndrome factors occurring in the body due to the exotoxins of infected and contaminated bacteria.

Results of the tissue growth factor study. Among the cytokines involved in the regulation of angiogenesis, vascular endothelial growth factor plays an important role. Among the cytokines of this class (VEGF), the most studied peptide is VEGF-A.

VEGF-A is a fundamental mediator of physiological and pathophysiological angiogenesis. In humans, VEGF-A consists of 165 amino acids, while in rats, its number is 1 less, i.e., VEGF-A164. Initially, VEGF was considered a factor that enhances the permeability of endothelial cells, but it was later proven to have a significant angiogenic effect, i.e., a significant effect on vascular growth in normal and pathological conditions.

In the blood of experimental animals, VEGF-A164 manifested with significant dynamic changes during the experiment (see Table 4).

Table 4.

Dynamics of changes in VEGF-A (pg/ml) levels in the blood plasma of experimental animals, M±m

Review days	Control group	After treatment	
		Comparison group	Main group
1-кун	1,14±0,038	2,44±0,084***	5,35±0,16$^{***\wedge\wedge\wedge}$
3-кун	2,2±0,071$^{\&\&\&}$	3,87±0,12$^{***\&\&\&}$	7,40±0,25$^{***\wedge\wedge\wedge\&\&\&}$
7-кун	2,5±0,078$^{\&}$	5,46±0,17$^{***\&\&\&}$	8,31±0,27$^{***\wedge\wedge\wedge\&}$
10-кун	2,14±0,068$^{\&\&}$	4,04±0,13$^{***\&\&\&}$	7,31±0,25$^{***\wedge\wedge\wedge\&}$
14-кун	0	2,04±0,065$^{\&\&\&}$	6,33±0,20$^{\wedge\wedge\wedge\&\&}$
21-кун	0	1,54±0,048$^{\&\&\&}$	5,41±0,17$^{\wedge\wedge\wedge\&\&}$

*Note: * - significance of comparison with control group indicators (*-P<0.05; **-P<0.01; ***-P<0.001); ^-reliability of comparison with comparison group indicators (^-P<0.05; ^^-P<0.01; ^^^-P<0.001). & - significance of comparison with indicators of previous days (& - P<0.05; &&-P<0.01; &&&-P<0.001)*

In the blood plasma of the control group rats, the VEGF-A value increased by 1.93 times (p<0.001) on the 3rd day of the experiment, indicating the onset of inflammation and angiogenesis. On the 7th day, it remained virtually unchanged and significantly exceeded the values of the intact group rats by 2.19 times (p<0.001). In the final days of the experiment, it did not differ from the indicators of the intact rats. We attributed this to the fact that the compensatory mechanism in the animals' bodies initially manifested as a slight increase in VEGF-A, and due to the absence of a stimulating factor, it equaled the values of intact rats.

In the blood plasma of rats in the comparison group with purulent wounds, the level of VEGF-A increased by 1.76 times on the 3rd day of the experiment (P<0.001); On the 7th and 14th days, it was 2.18 (P<0.001) and 2.04 (P<0.001) times higher than the control group rats, respectively. We attributed this to the fact that the ointment (levomecol) applied to the wound defect of the animals in the comparison group had a positive effect on the reparative processes in the wound.

It is known that VEGF-A can also manifest as an increase in its level in the blood as a primary marker against the background of ischemia in the animal body. The increase in its level (VEGF) in the control group rats may be influenced not only by compensatory mechanisms but also by the state of ischemia on the wound surface against the background of purulent inflammation of the soft tissues.

The level of VEGF-A in the blood plasma of experimental animals with purulent wound defects in the main group was significantly higher on the 3rd day of the experiment compared to the comparison group and the control group, by 1.91 (P<0.001) and 3.36 times (P<0.001), and by the 7th and 14th days by 1.52 (P<0.001) and 3.32 (P<0.001) times, respectively; 3.10 (P<0.001) and 6.33 (P<0.001) times (see Fig. 4).

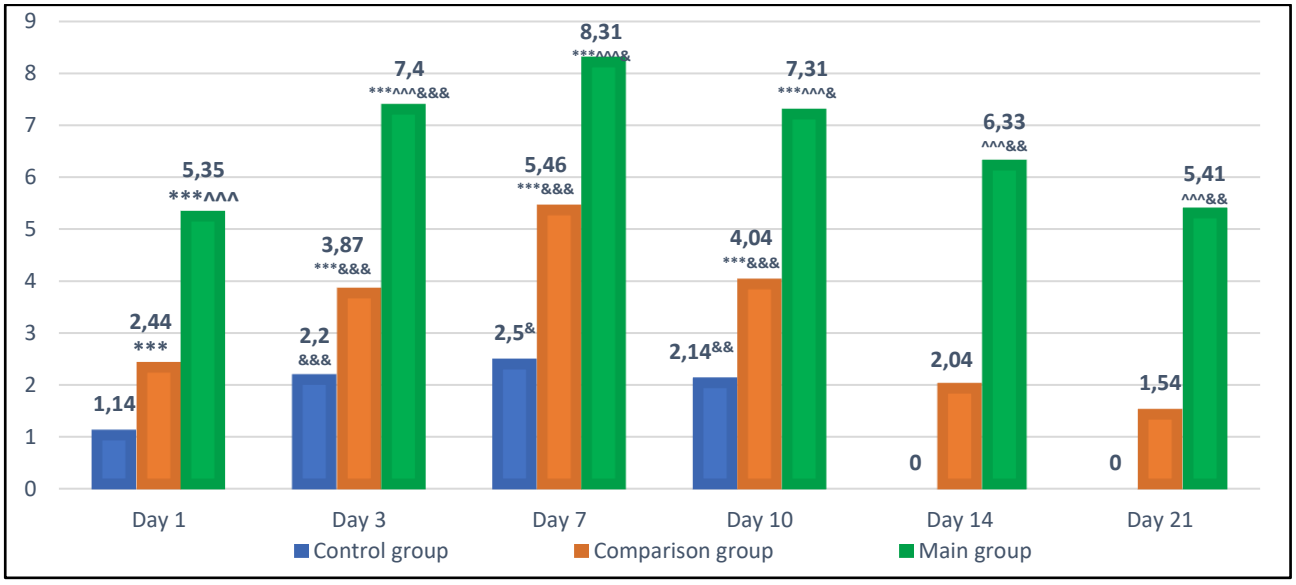


Figure 4. **Dynamics of VEGF-A levels (pg/ml) in the blood of animals in the primary group using phyllalbin gel-like collagen**

*Note: * - significance of differences with the control group indicators (***-P<0.001); ^- significance of differences with comparison group indicators (^^^-P<0.001). & - significance of the difference with the indicators of the previous days (& - P < 0.05; &&-P<0.01; &&&-P<0.001)*

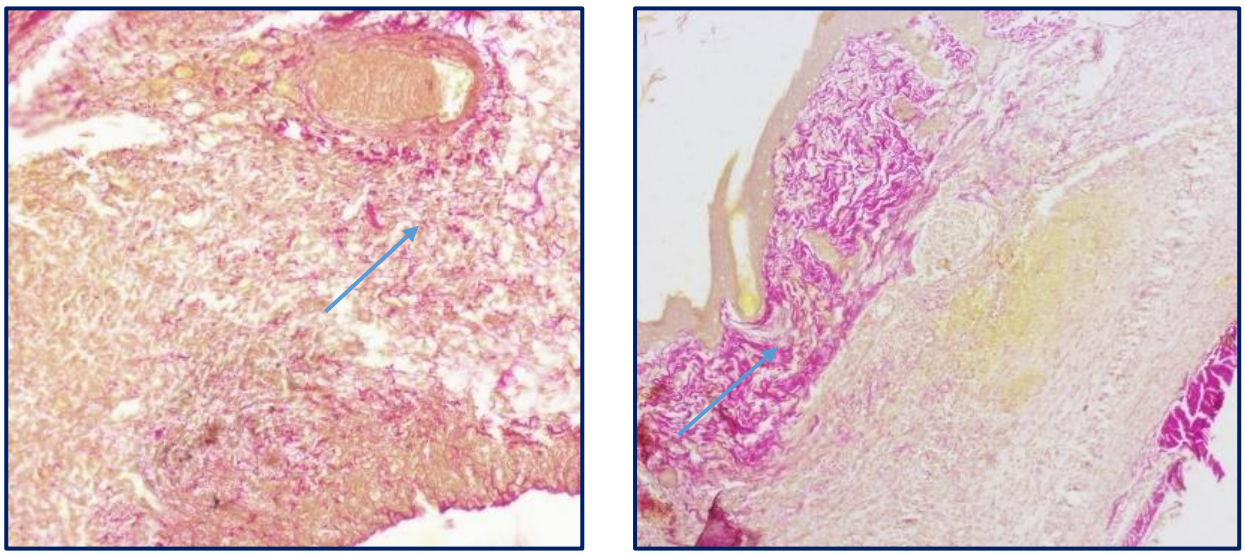
The increase in the level of VEGF-A in the blood of the control group animals and its value in the final days of the experiment, similar to that of intact rats, depends on the intensity of the injury. A statistically significant increase in the level of VEGF-A in the rats of the main group compared to the comparison and control groups was manifested by the effect of the gel-like collagen with filllabin used by us on the role of angiogenesis in the formation of new tissue by stimulating regenerative and reparative processes of wound healing.

Results of pathomorphological studies. Seven days after the injury, signs of acute inflammation in the tissue intensified. Inflammatory cell infiltration remained in the wound area, but a significant increase in the number of neutrophils and an increase in the number of macrophages were observed, indicating the progression of acute inflammatory symptoms. There are foci of necrosis, but they are much larger than in previous days. The purulent secretion consists of a large amount of cellular debris. Inflammation in the injured areas remains pronounced, but neutrophils are found in a more pronounced form. There are signs of angiogenesis, i.e., the formation of new blood vessels, which helps improve the delivery of nutrients to the wound area.

In the rats of the control group, we also stained the tissue biopsy of the purulent wound with Van Gieson to see the effect of collagen-fillalbin gel. According to the Van Gieson dye, the collagen fibers were stained bright red, indicating the presence of fibrin and new fibers. Collagen fibers in the wound area are irregular; they are represented by a loose, disorganized network, which is the initial stage of the process (see Fig. 5a). In some places, a denser accumulation of collagen fibers can be seen, which may be associated with the formation of wound lining and an attempt to fight infection. Collagen deposition is primarily observed in the deep part of the wound, indicating that granulation

tissue has begun to form. This is a normal situation where the body begins to lay the groundwork for healing, but the process is not yet complete. Significant vascularization is observed in the wound area-new capillaries are formed in the granulation tissue, which facilitates the delivery of oxygen and nutrients for recovery. Inflammatory cells, such as neutrophils, actively penetrate the area of injury, helping to destroy infected and dead cells. However, on the 7th day, the inflammation is still pronounced.

On the 10th day of the study, an increase in the amount of collagen fibers is observed, and they begin to occupy the surface of the purulent wound. The central part of the wound is further organized by collagen fibers, indicating that a strong structure of the scar has begun to form. Nevertheless, the presence of irregular, loose fibers was found in some areas, which is characteristic of the ongoing healing process. In areas of active regeneration, the tissue has a denser structure and a cohesive layer of collagen fibers is formed, indicating the formation of a scar that increases tissue strength over time. The granular tissue continues to develop. It becomes more mature, and specific fibroblasts and new capillaries appear within it, contributing to the tissue healing process and wound healing (see Fig. 5b). Overall, the tissue continues to recover progressively on the 10th day. With the reduction of inflammation, the increase and formation of collagen fibers, the process of granulation tissue and healing continues, and the wound enters an early stage of healing, with clear signs of tissue strengthening and healing.



a b

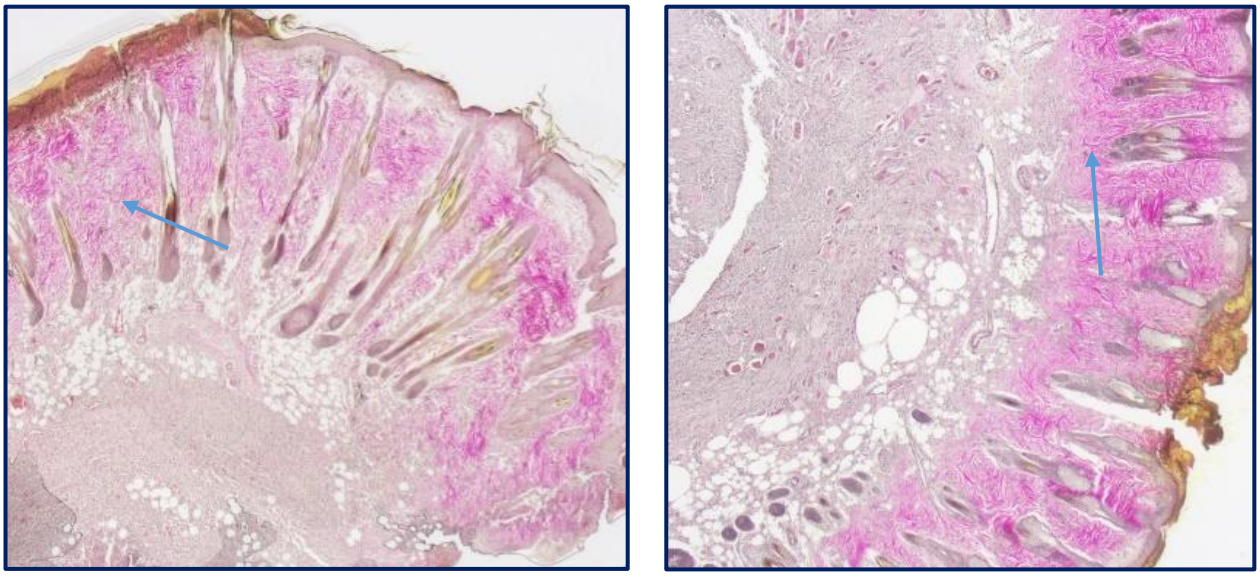
Figure 5. **Control group. A model of a purulent soft tissue wound. Purulent tissue. Day 7. Collagen fibers in the wound area are irregular; they are represented by a loose, disorganized network (Fig. a, Van Gieson. X20). Day 10. In areas of active regeneration, the tissue has a denser structure and a continuous layer of collagen fibers is formed (Fig. b, Van Gieson. x20)**

On the 7th day of the comparison group, due to local treatment with the antiseptic drug "Levomekol" ointment, the purulent process in the rat's interscapular wound led to earlier regeneration processes and a reduction in infection contamination compared to the control group.

During Van Gieson staining of purulent tissue obtained from rats in the comparison group on the 7th day of the experiment, an increase in the number of collagen fibers in the connective tissue matrix was observed on the 7th day of treatment, and they began to form more frequently. However, this web is not yet fully mature, and the collagen fibers are loose in some places, which is characteristic of the early healing stage. The presence of both organized and structured collagen fibers was noted in the wound area, indicating the ongoing process of tissue remodeling (see Fig. 6a).

Granulation tissue actively develops a network of microvasculature, which helps improve the blood supply to the wound. The appearance of fibroblasts and young vessels in these areas indicates that the tissue recovery process is ongoing. No signs of scarring were observed in some areas of the wound. Collagen fibers begin to fill the damaged areas, contributing to tissue strengthening and reducing the size of the wound. Collagen fibers begin to actively form and become organized. Granulation tissue continues to develop, and inflammation has significantly decreased. Scarring began and a gradual closure of the wound was noted.

When using the treatment method with Levomekol ointment, a significant improvement in the condition of the purulent wound site in the tissues of the experimental group was observed on the 10th day of treatment. The healing process continued to develop, and signs of tissue regeneration and a decrease in the inflammatory response were clearly manifested. Against the background of Van Gieson staining, collagen fibers acquire a bright red color, indicating their maturity and active regeneration process. Collagen synthesis continues in the wound area, and the collagen itself is a dense and organized network. This indicates progressive collagenation, which forms the basis for the final formation of the scar. Collagen fibers in the wound area become dense, forming a more mature and structured network (see Fig. 6b). The process of vascularization continues in the peripheral parts of the wound, which is characteristic of the recovery stages that facilitate the delivery of nutrients and oxygen. Moderate vascular hyperplasia was also noted in these areas. Despite clear signs of regeneration, areas of cellular infiltrate remain in the tissue, indicating an inflammatory process; however, its severity significantly decreased by the 10th day compared to previous stages. Necrotic zones were also reduced, which confirms the active development of restorative and reparative processes; compared to day 7, a significant reduction in the zone of necrosis and an improvement in tissue structure were observed on day 10. The entire healing process shifts toward the formation of a mature scar, indicating a higher level of recovery compared to previous stages. Thus, treatment using levomecol on the 10th day led to a significant improvement in wound healing, improved collagenation, and a reduction in inflammatory processes.



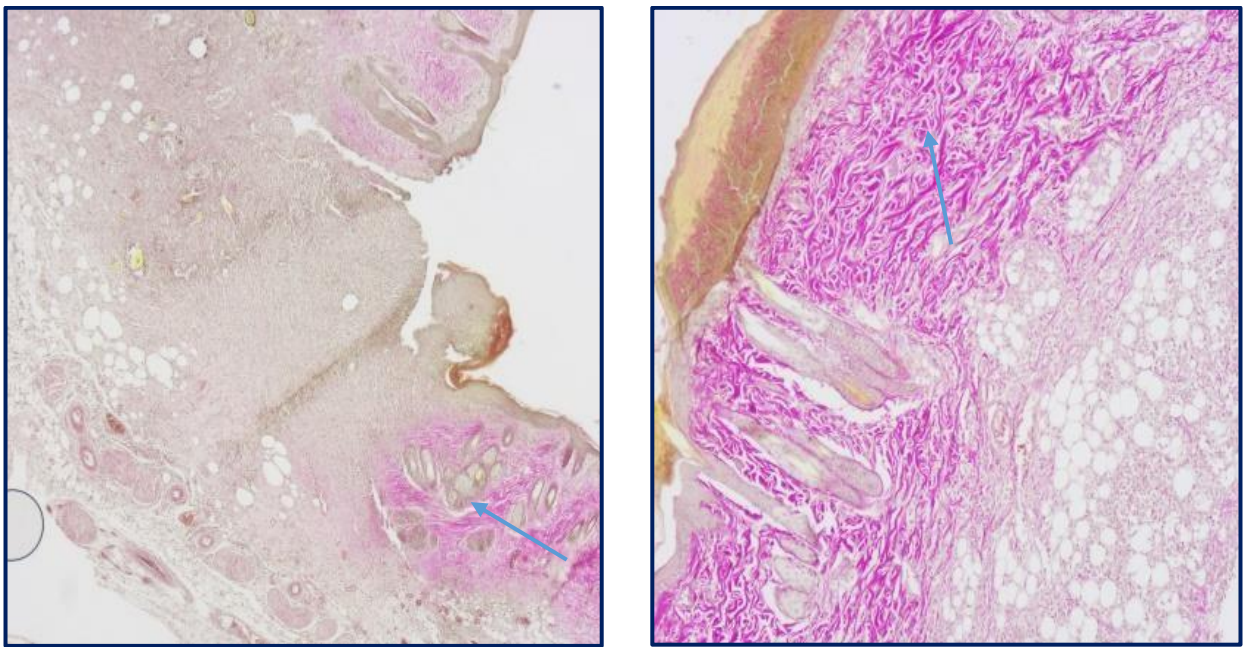
a b

Figure 6. **Comparison group. A model of a purulent soft tissue wound. Purulent tissue. Day 7. Active development of a network of microvessels in granulation tissue (Fig. a, Van Gieson x4). Day 10. Collagen fibers in the wound area have become dense, forming a more mature and structured network (Fig. b, Van Gieson. x20)**

In the primary group of rats treated with the collagen phyllalbin gel form, the tissue of the interscapular purulent wound showed the first signs of tissue regeneration 7 days after treatment. A high degree of changes associated with tissue healing and regeneration was observed in the wound area.

To analyze the pathohistological state of the purulent tissues of the main group of rats with a purulent wound process on the 7th day of treatment with a collagen gel form using Van Gieson dye, the following changes can be noted: the structure of the collagen fibers reached maturity. In some places, they are immature, with weakly oriented fibers, indicating an early process of connective tissue formation; however, the amount of cells and fluid in the intercellular space has decreased. In some areas, active growth of new vessels (angiogenesis) is observed, which confirms the process of regeneration with synthesis, and the formation of granulation tissue has a high trend. This is also a sign that the gel form of phyllalbin-containing collagen acts to accelerate tissue regeneration by stimulating blood supply (see Fig. 7a). It is evident that collagen fibers participate in the process of strengthening tissue structures. However, in some parts of the gel, signs of its complete decomposition can be seen, which reflects the fact that the material's absorption process has reached a high level.

It can be seen that significant changes in the cellular composition of the tissue, as well as in the area of tissue regeneration, have been completed in the model of purulent wounds in rats of the main group treated using the gel form of collagen with phyllalbin for 10 days of our study. Significant changes in collagen fibers were observed in all tissues, as confirmed by Van Gieson staining. Increased collagenation is observed in the affected area, manifesting as intensive red staining of the collagen fibers. At the same time, the structure of these fibers appears more orderly and less chaotic than in the early stages of recovery. Specifically, collagen fibers are formed in the form of dense fibrils, indicating the final stage of scarring (see Fig. 7b). The environment around collagen fibers is characterized by the presence of fibroblasts and inflammatory cells, such as macrophages and lymphocytes. With tissue regeneration, the number of these cells decreases significantly. This indicates the transition of acute inflammation into a more chronic stage, which is characteristic of improved recovery. Active blood circulation is observed, and the number of native formations near the wound area has increased. Many new capillaries, as well as vessels containing red blood cells, indicate that blood supply has been restored. When comparing the image data with samples on day 7, we can see that the wound has healed on day 10, and we confirm this. With phyllalbin, collagen exhibits more pronounced and effective collagenization, indicating progressive recovery. On the 10th day of treatment using the collagen gel form of phyllalbin, we observed that collagenation reached its final stage and tissue recovery improved significantly compared to previous stages.



a b

Fig. 7. **The main group. A model of a purulent soft tissue wound. Purulent tissue. Day 7: Active growth of new vessels (angiogenesis) is observed in certain areas, indicating a high trend toward the synthesis and regeneration process (Fig. a, Van Gieson. X20)**

Discussion. Comparison of biochemical studies. Thus, in the primary group of rats treated with biomedical-engineered collagen-containing gels for purulent wounds formed in the soft tissue between the scapulae, changes in the dynamics of systemic inflammatory syndrome factors (IL-1β, IL-2, IL-10) and procalcitonin levels were recorded. These cytokines were studied to determine the course of the purulent process and the principles of their influence on the proliferation process after treatment.

Furthermore, based on the results of the complete blood count, the leukoformula, leukocyte intoxication index, and neutrophil response were determined. In the initial stages of the study, a decrease in immune system decompensation and subsequent purulent-inflammatory processes was noted, along with the formation of immunity against the background of IL-2.

On the 7th day, the level of inflammatory IL-1β reached its highest critical point, 0.142±0.00 pg/ml. In IL-2 and IL-10, a decrease was observed compared to the comparison group, amounting to 0.364±0.01 pg/ml and 0.155±0.00 pg/ml, respectively. The results obtained proved the positive and desirable effect of phyllalbin gel-like collagen on immunomodulatory properties and wound healing activity compared to levomecol.

Procalcitonin, being a highly sensitive cytokine to inflammation, manifested itself by the introduction of bacterial exotoxin into the blood and remained at a critical high level only from 24 to 36 hours, i.e., 1.1±0.24 ng/ml. In the following days, after the onset of the purulent process in the wound, interleukins began their activity, and the procalcitonin level decreased dynamically, reaching 0.38±0.77 ng/ml by day 21.

The results obtained by us showed that the new local phyllalbin gel-like collagen is an important complex treatment method in the treatment of purulent wounds, is a modern treatment method that leads to faster wound healing, and it has been proven that hematological, biochemical, immunological and bacteriological indicators give good results on average 4 days earlier than the comparison group.

Comparison of the effect on purulent soft tissue wounds.

Significant inflammation, necrosis, and tissue infiltration around the wound defect were observed in the rats of the control group. On the 7th and 10th days, the purulent process persisted and intensified, the wound widened, inflammatory processes intensified, and a pronounced fibrous process began; the wound persisted until the 21st day but did not heal completely.

Due to the lack of treatment in dynamics, this led to soft tissue deterioration, severe inflammation, and necrosis, which slowed down healing processes and contributed to the development of the wound defect.

On the 7th day of the comparison group, the antiseptic and reparative effect of levomekol significantly reduced inflammatory reactions, reduced exudation, and promoted the onset of tissue regeneration during topical treatment. By the 10th day, the soft tissues had practically recovered, inflammatory reactions were minimal, and wound epithelialization was not complete.

In the purulent wound of the soft tissues between the scapulae of rats in the main group, inflammatory factors decreased by the 7th day, and rapid wound healing and partial tissue restoration occurred. Fillalbin stimulated bactericidal action, resulting in a decrease in the amount of bacterial infection, while collagen increased its effect, improved microcirculation, and exerted antiseptic and reparative effects. On the 10th day, the inflammatory processes almost completely disappeared, ulcers decreased, tissue regeneration intensified, the wound healed completely, and epithelialization was completed.

Conclusion. A stable experimental model of a purulent wound was created by placing a swab impregnated with an autocal suspension into the soft tissue under a special "bridge" on an open wound formed between the scapulae of experimental rats. In the process of wound proliferation and regeneration, the normalization of cytokine levels (IL-1β, IL-2, IL-10, procalcitonin) and the maintenance of a high level of tissue growth factor (VEGF-A) (5.41±0.67 pg/ml) were established in the study group during treatment with biomedical cellular coating material. Pathomorphological analysis showed that as a result of treatment with the biomedical cell liner, microcirculation and regeneration processes began in the primary group on average 6.9±1.2 days earlier than in the comparison group and on average 10.7±1.5 days earlier than in the control group.

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