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# COMPLEX BIOSTIMULATION OF INTRAPLEURAL ADHESIOGENESIS IN THORACAL SURGERY

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**The aim of the study** is to determine the effectiveness of the use of platelet enriched plasma in the complex treatment of chest trauma and chronic pleural empyema.

**Materials and methods.** The work was performed on 450 male rats, simulated with chest trauma (n=180) and chronic pleural empyema (n=270). In the experimental groups, biostimulation of adhesiogenesis as an intrapleural injection of 1 ml of platelet-enriched plasma was carried out; in the comparison group; the animals with pleural empyema were injected with 1 ml of doxycycline solution; in the negative control groups, the treatment was not carried out at all. Withdrawal from the experiment took place on the 10th, 20th, 30th days. The samples of intrapleural adhesions were fixed in 10% formalin, followed by histological tracing and preparation of micropreparations, staining with hematoxylin and eosin. The morphometric study included determination of the volume fraction (VF) of collagen and reticular fibers; fibrin; inflammatory cells; bloodstream (%).

**Results.** An intrapleural administration of platelet-rich plasma is an effective way to stabilize the rib cage in chest injuries, and to eliminate residual cavities in chronic pleural empyema. When assessing the severity of the adhesions in chest trauma, it was found out that adhesions are most often visualized at the sites of rib fractures (from 13.3 to 40%). In pleural empyema, during the entire process of observation, the VF of collagen fibers forming adhesions was higher in the group with biological stimulation of adhesiogenesis than in the NCpe group and in the CG. In the PRP group, already at the initial stages of the experiment, this indicator was significantly lower than in the NC and CG ($p<0.05$).

**Conclusion.** Based on the data obtained, the effectiveness of the use of platelet-enriched plasma in thoracic surgery for the biological potentiation of adhesiogenesis has been proved in experimental chest injuries and chronic pleural empyema.

**Keywords:** thoracic surgery; biostimulation of adhesiogenesis; platelet-enriched plasma; chest trauma; pleural empyema

**Abbreviations:** Ch.Inj.T – chest injury trauma; NCt – negative control group with chest trauma (without pharmacological correction); PRPt – administration of platelet-rich plasma for the treatment of chest trauma; PE – pleural empyema; NCpe – negative control group with pleural empyema (without pharmacological correction); PRPpe – administration of platelet-rich plasma for the treatment of pleural empyema; CG – comparison group, CGpe – comparison group without pharmacological correction.

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# КОМПЛЕКСНАЯ БИОСТИМУЛЯЦИЯ ВНУТРИПЛЕВРАЛЬНОГО АДГЕЗИОГЕНЕЗА В ТОРАКАЛЬНОЙ ХИРУРГИИ

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**Цель.** Определить эффективность применения плазмы, обогащенной тромбоцитами в комплексном лечении травмы грудной клетки и хронической эмпиемы плевры.

**Материалы и методы.** Работа выполнена на 450 крысах-самцах, которым моделировали травму грудной клетки (n=180) и хроническую эмпиему плевры (n=270). В опытных группах осуществлялась биостимуляция адгезиогенеза: внутриплевральное введение 1 мл плазмы, обогащенной тромбоцитами, в группе сравнения при эмпиеме плевры вводили 1 мл раствора доксициклина, в группах негативного контроля лечения не проводилось. Выведение из эксперимента на 10-е, 20-е, 30-е сутки. Образцы внутриплевральных сращений фиксировали в 10%-ом формалине с последующей гистологической проводкой и изготовлением микропрепаратов, окраской гематоксилином и эозином. Морфометрическое исследование включало определение объемной доли (ОД) коллагеновых и ретикулярных волокон; фибрина; клеток воспалительного ряда; сосудистого русла (%).

**Результаты.** Внутриплевральное введение плазмы, обогащенной тромбоцитами, является эффективным способом стабилизации реберного каркаса – при травмах грудной клетки, и ликвидации остаточных полостей – при хронической эмпиеме плевры. При оценке выраженности спаечного процесса при травме грудной клетки установлено, что наиболее часто спайки визуализируются в местах перелома ребер (от 13,3 до 40%). При эмпиеме плевры на протяжении всего наблюдения ОД коллагеновых волокон, формирующих спайки, была выше в группе с биологической стимуляцией адгезиогенеза, чем в группе НКэп и в ГСэп. В PRP-группе данный показатель уже на начальных сроках эксперимента был достоверно ниже, чем в группе НК и ГС ($p<0,05$).

**Заключение**. На основании полученных данных доказана эффективность применения плазмы, обогащенной тромбоцитами в торакальной хирургии для биологического потенцирования адгезиогенеза: при экспериментальных травмах грудной клетки и хронической эмпиеме плевры.

**Ключевые слова:** торакальная хирургия; биостимуляция адгезиогенеза; плазма, обогащенная тромбоцитами; травма грудной клетки; эмпиема плевры

**Сокращения:** ТГК – травма грудной клетки; НКт – группа негативного контроля с травмой грудной клетки (без фармакологической коррекции); PRPт – введение плазмы, обогащенной тромбоцитами для лечения травмы грудной клетки; ЭП – эмпиема плевры; НКэп – группа негативного контроля с эмпиемой плевры (без фармакологической коррекции); PRPэп – введение плазмы, обогащенной тромбоцитами для лечения эмпиемы плевры; ГС – группа сравнения; ГСэп – группа сравнения без фармакологической поддержки.

## INTRODUCTION

At present, one of the promising directions of regenerative medicine is biological stimulation of repair processes on the basis of platelet rich plasma (PRP) containing numerous growth factors and cytokines [1–3].

The drugs developed on the basis of autologous components, are increasingly being introduced into cosmetology and traumatology. However, the biotechnology based on PRP technologies, has not yet found application in thoracic surgery which determines the relevance of this work.

The problems of the rib cage stabilization in severe concomitant injuries and the elimination of residual cavities in chronic pleural empyema, are still unresolved. Such problems require search for new solutions on the basis of biological stimulation of adhesiogenesis in the complex treatment of these nosologies [4–7].

**THE AIM** of the study is to determine the effectiveness of the use of platelet enriched plasma in the complex treatment of chest trauma and chronic pleural empyema.

## MATERIALS AND METHODS

### Experimental animals

The experiment was carried out on 450 nonlinear sexually mature male rats (confluence), weighing 280–300 g, which were kept under standard vivarium conditions, with a natural change of the daily cycle, free access to extruded feed and water. The maintenance and manipulations were carried out in accordance with the order of the Ministry of Health of the USSR No. 755 dated 08/12/1977 and the European Convention for the Protection of Vertebrate Animals used for Experiments or for Other Scientific Purposes (Strasbourg, March 18, 1986) [8, 9]. A positive conclusion on experimental studies of the Local Independent Ethical Committee of Volgograd State Medical University was received on September 29, 2016, Protocol No. 12 – 2016.

### Study Design

The design of the experiment is shown in Figure 1.

The animals were modeled for chest trauma (n=180) and chronic pleural empyema (n=270) using the authors' post-anesthesia techniques (chloral hydrate 350 mg/kg intraperitoneally). When modeling Ch.Inj.T, a negative control group with chest injury trauma (NCt) and an experimental group (PRPt) were isolated, then underwent biological potentiation of adhesiogenesis by introducing platelet-rich plasma (PRP) into the pleural cavity: a set for blood sampling Plasmolifting™, Ltd Plasmolifting, Kazan, Russia; TU 9437-002-27837594-2015, registration certificate No. RZN 2016/3980 dated 04.19.2016. The protocol of the procedure was as follows:

Stage I. Blood sampling in the volume of 5 ml from the tail vein using a syringe, into a specialized Plasmolifting™ tube.

Stage II. Centrifugation 1000 G (3200 revolutions) for 5 min., separation into fractions, obtaining platelet autoplasm.

Stage III. Supernatant sampling (thrombotic autologous plasma), located in the upper part of the tube above the separation gel (Fig. 2Б).

Stage IV. Injection of 1 ml of the drug in fractured ribs: pointwise into the fracture zone, subpleurally into the pleural cavity; directly into the residual pleural cavity – in chronic pleural empyema.

In addition to the above-described groups, in the simulation of chronic PE, a comparison group (CGpe) was added, the animals of which were injected intrapleurally with 1 ml of a doxycycline solution. Doxycycline (Doxycyclinum) is a semisynthetic bacteriostatic antibiotic from the group of tetracyclines with a broad spectrum of activity. Its chemical name is (4S, 4aR, 5S, 5aR, 6R, 12aS)-4-dimethylamine-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxy-1,4,4a,5,5a,6,11,12a-octahydronaphthacene-2-carboxyamide monohydrochloride, mixed with ethanol (2: 1), hemihydrate $C_{22}H_{24}N_2O_8$*HCl*½$C_2H_5OH$*½$H_2O$. Its invented name is Doxycycline, BINERGIYA Ltd (Russia), lyophilisate for preparation of solution for infusions 100 mg, packed in vials (5) of contoured plastic (1), in cardboard packs. The code is ATX J01AA02. Its composition is: doxycycline (in the form of hydrochloride) 100 mg; the excipients are: sodium disulfite – 6 mg, disodium edetate – 0.02 mg. In addition to the antibacterial effect of Doxycycline, this substance, according to the National Clinical Guidelines for Thoracic Surgery, is the drug of choice for chemical pleurodesis [10-12]. Withdrawal from the experiment was carried out on the 10th, 20th and 30th days of the experiment. The description of the macroscopic picture of the pleural cavity and the collection of intrapleural adhesions was carried out with a maximum preservation of the intrinsical points [13].

### Histological study

1350 samples of intrapleural adhesions were taken with a maximum preservation of the intrinsical points of adhesions between the parietal and visceral pleura and fixation of the material in a 10% solution of neutral formalin (the exposure for 24 h); a standard histological processing was carried out, passing the fixed material through ethyl alcohol in the ascending concentrations (from 70° up to 100°) and chloroform. A comprehensive morphological study included the production of serial sections of intrapleural adhesions at different periods of the experiment with staining with hematoxylin and eosin. Micrographs were made using a LeicaDM 100 microscope with a digital camera, magnification ×100, ×200, ×400.

### Morphometric study

A morphometric study was carried out in accordance with the established principles of quantitative morphological investigations, according to which the measurements are carried out on microphotograms obtained by photographing serial sections (and determining the number of objects on at least 10 glasses in 10 fields of view) [avtandilov]. The Videotest Morpho software was used to determine the volume fraction (VF) of collagen and elastic fibers, fibrin, cellular composition of adhesions (lymphocytes, leukocytes, fibroblasts) and VF of the blood-stream [14].

### Statistical processing of results

The results were processed using the STATISTICA 7.0 software package (StatSoft, USA), (M±SEM were determined), Wilcoxon's nonparametric test, Student's test and confidence index (p). The results were considered reliable at $p<0.05$.

## RESULTS

### Efficiency of platelet-rich plasma in intrapleural administration for potentiating adhesiogenesis in chest trauma according to the results of macroscopic examination

As a result of a comparative analysis of the experimental group and the negative control group, it was found out that on the 10th day of the experiment with chest injury trauma, there were no cases of consolidation of rib fractures. In the negative control group with chest trauma

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(without pharmacological correction), the adhesion process was absent in 16.7% of cases; in 36.7% the adhesions were formed locally, exclusively in the area of the surgical trauma, and were represented by whitish translucent cords (arachnoid adhesions), Figure 2A.

When PRP was injected intrapleurally, adhesions were not formed only in 6.7%, which was significantly less than in the negative control group with chest trauma (without pharmacological correction) ($p<0.05$). The fact that intrapleural adhesions were represented by denser (filmy) adhesions, localized mainly between the thoracotomy scar and the parietal and visceral pleura, is of great importance (Fig. 2B).

On the 20th day of the experiment, consolidation of rib fragments was recorded in 66.7% of the animals in the negative control group with chest trauma (without pharmacological correction) and in 77.8% in the experimental group. Adhesions in the negative control group with chest trauma (without pharmacological correction), as in the previous period of the experiment, were predominantly formed in the area of surgical trauma (in 43.3%) and were represented by arachnoid and membranous adhesions (Fig. 3A).

When platelet-rich plasma (PRPt) was injected into the pleural cavity on day 20, adhesions were absent only in 1 case (3.3%), in other cases adhesion was recorded in 13.3% (4 cases) with total pleural cavity filling. The most frequently detected adhesions were in the fracture zone (40.0%), there were also single local adhesions (20.0%) and single adhesions outside the fracture zone (23.3%). The adhesions were represented by dense, planar adhesions (Fig. 3B).

On the 30th day of the experiment, the negative control group with chest trauma (without pharmacological correction) showed consolidation of fracture sites with the formation of a pronounced callus. Narrowing and deformation of the intercostal spaces, formed due to the adhesive process, was noted. No total obliteration of the pleural cavity was detected (Fig. 4A).

By the end of the experiment (day 30) in the PRPt group, the total pleural cavity obliteration was observed in 13.3% (4 cases). Figure 4B shows the most typical situation characterized by the formation of a planar intimate adhesion at the fracture site during biostimulation of adhesiogenesis with platelet-rich plasma. The adhesion length was 10 mm, the width – 4 mm, thickness – up to 1 mm, the total area of the organ adhesion was 40 $mm^2$; there was a depletion of vascularization compared with the previous period.

Thus, a comparative analysis of the adhesion process in the pleural cavity with chest trauma revealed significant differences in NCt rats and the PRPt experimental group. In the NCt group, arachnoid and filmy adhesions prevailed, while biostimulation of adhesiogenesis with platelet-rich plasma revealed ribbon-like and planar adhesions, the density of which increased in the dynamics of the experiment.

### Efficiency of platelet-rich plasma when administrated intrapleurally, for potentiating adhesiogenesis in chest trauma according to the of histological study results

On the 10th day of the experimental chest trauma, histological examination of pleural adhesions showed the predominance of chaotically located connective tissue fibers with a pronounced leukocyte infiltration in both – the NCt group and the PRPt group. At the same time, in the studied samples of the NCt group, diffusely located serous-hemorrhagic exudate with fibrin concretions was found (Fig. 5A).

On the 10th day of the experiment, thin fibers of connective tissue, located unevenly, mainly in the zone of fracture and drug administration, were determined in the experimental group. The amount of exudate was insignificant, its organization was notified against the background of a persisting inflammatory reaction (Fig.5B).

On the 20th day of the experiment, the volume of serous-hemorrhagic exudate in the NCt group was insignificant; the edema of the connective tissue, represented by chaotically localized fibers, by the type of myxomatous transformation, was revealed. Single vessels and diffuse, predominantly neutrophilic, inflammatory infiltration were identified. There was a discrepancy between immature pevral adhesions and the time of the experiment (Fig. 6A).

When PRP was adminisrated on the 20th day, young pleural adhesions containing fibers oriented parallel to the surface of the walls of the pleural cavity, were determined. In loosely located connective tissue fibers, single hemosiderin granules were visualized. It was associated with a change in the permeability of the endothelium and basement membrane and indicated continuing adhesiogenesis (Fig. 6B).

On the 30th day of the experiment, adhesions were still immature in the NCt group. Vascular filling and the presence of hemosiderin granules with siderophages indicated ongoing angiogenesis and the formation of pleural adhesions. The vessels were thin-walled, full-blooded and located unevenly among the fibers of the connective tissue. The presence of single lymphocytes, plasma cells and neutrophils was detected, as well as an abundance of macrophages (siderophages) (Fig. 7A).

By the end of the experiment, mature adhesions in the area of the rib fracture had been revealed. It took place in histological examination of morphological changes in the pleural cavity during biostimulation of adhesiogenesis by the introduction of platelet-rich plasma. The adhesions were formed by strictly oriented collagen fibers and included fibroblasts, fibrocytes, with single vessels in the field of view (Fig. 7B).

### Morphometric assessment of the effectiveness of platelet-enriched plasma for potentiating adhesiogenesis in dynamics of chest trauma

A morphometric analysis was completely consistent with the results of histological examination (Table 1).

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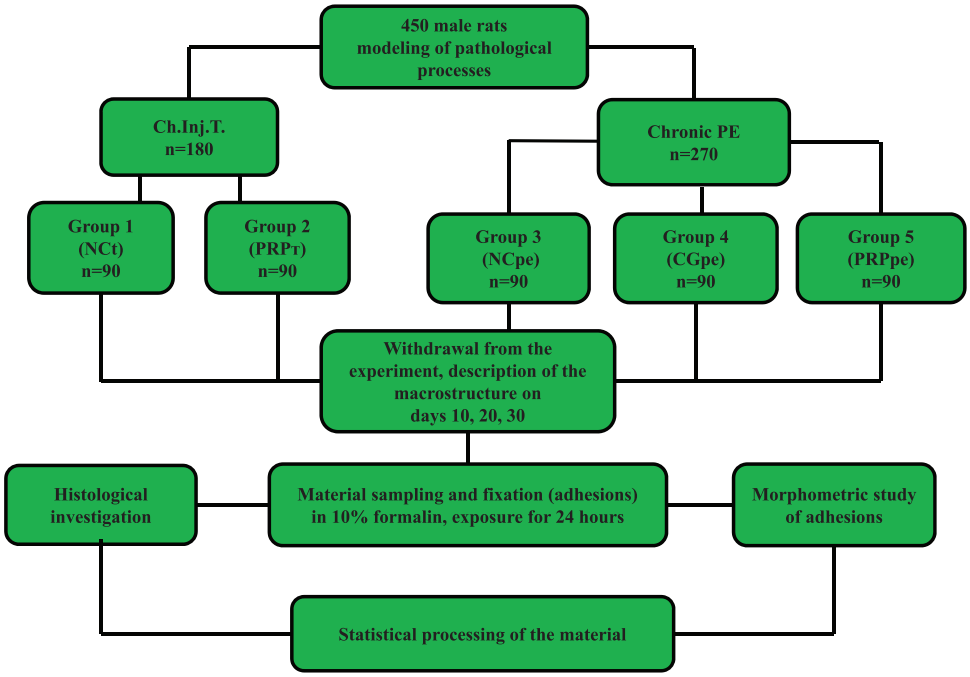
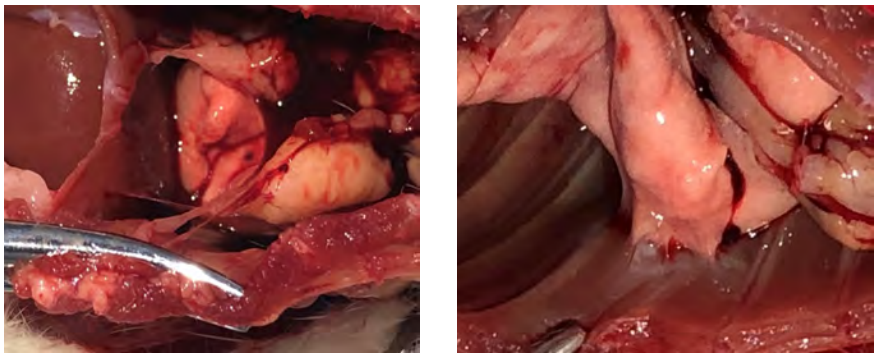



**Figure 1 – The design of the experiment**

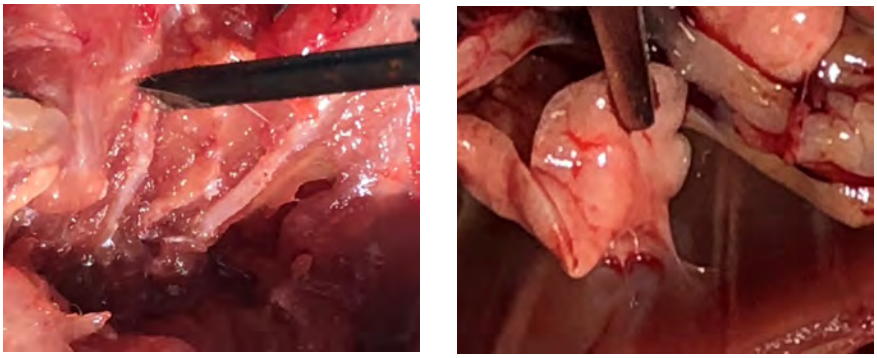
Notes: Ch.Inj.T – chest injury trauma; NCt – negative control group with chest trauma (without pharmacological correction); PRPt – administration of platelet-rich plasma for the treatment of chest trauma; PE – pleural empyema; NCpe – negative control group with pleural empyema (without pharmacological correction); PRPpe – administration of platelet-rich plasma for the treatment of pleural empyema.



**A** **B**

**Figure 2 – Pleural cavity on the 10th day of experimental chest injury**

Note: A. Negative control group. B. Experimental group. Administration of platelet-rich plasma (PRPt). Lack of consolidation of rib fragments.



**A** **B**

**Figure 3 – Pleural cavity on day 20 of experimental chest injury**

Note: A. Negative control group. The presence of consolidation of rib fractures, the absence of intrapleural adhesions. B. Experimental group. Administration of platelet-rich plasma (PRPt), formation of pleural adhesions.

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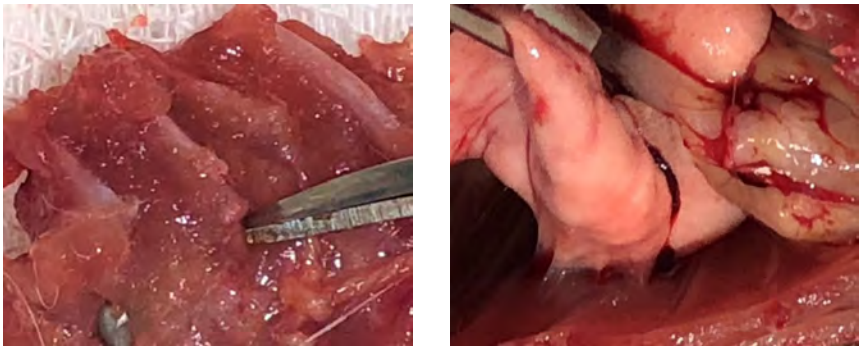
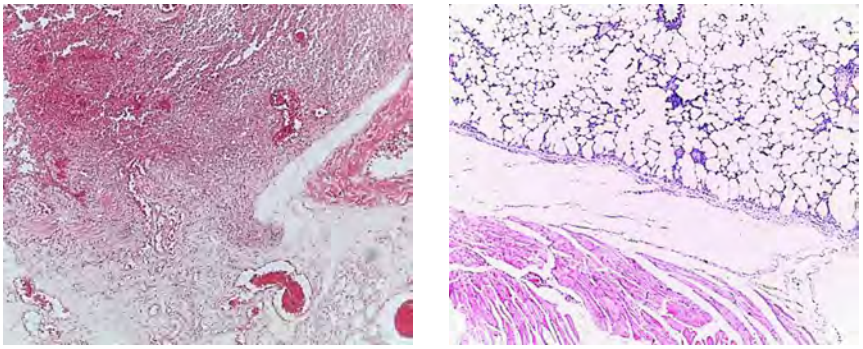


A B

**Figure 4 – Pleural cavity on day 30 of experimental chest injury**

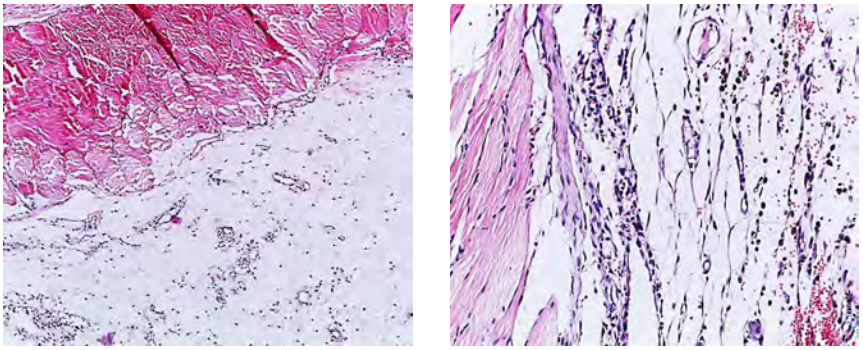
Note: A. Negative control group. The presence of consolidation of rib fractures, the absence of intrapleural adhesions. B. Experimental group. Administration of platelet-rich plasma (PRPt). Formation of plane adhesions.



A B

**Figure 5 – Experimental chest injury on day 10**

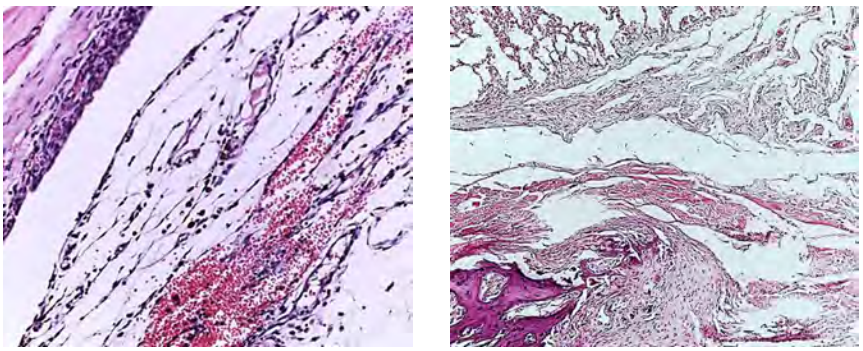
Note: A. NCt group. B. PRPt group. Staining with hematoxylin and eosin. Magnification ×400.



A B

**Figure 6 – Experimental chest injury on day 20**

Note: A. NCt group. Staining with hematoxylin and eosin. Magnification ×100. B. Experimental group PRPt. Staining with hematoxylin and eosin. Magnification ×200.



A B

**Figure 7 – Experimental chest injury on day 30**

Note: A. Group NCt. Staining with hematoxylin and eosin. Magnification ×200. B. Experimental PRPt group. Staining with hematoxylin and eosin. Magnification ×400.

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**Table 1 – Morphometric indices of the composition of adhesions in rats with chest trauma against the background of platelet-rich plasma, for potentiation of adhesiogenesis (M±m, %)**

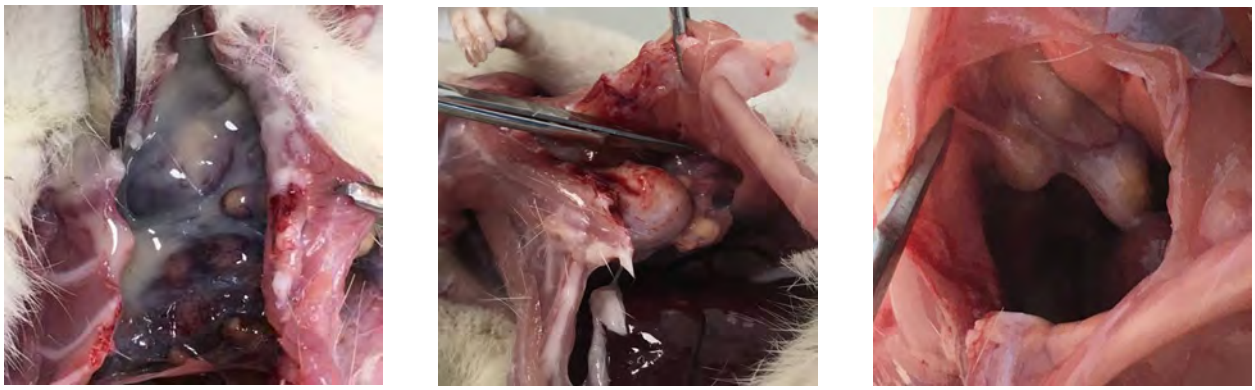
| Indices, VF (%) | Experimental groups | | | | | |
|---|---|---|---|---|---|---|
| | NCt | | | PRPt | | |
| | Day 10 | Day 20 | Day 30 | Day 10 | Day 20 | Day 30 |
| Collagen fibers | 3.04±0.8 | 15.11±2.3# | 33.72±9.7# | 8.23±0.9* | 29.15±3.1*# | 37.23±8.3*# |
| Reticular fiber | 31.56±3.5 | 25.05±0.9# | 2.95±0.7# | 30.19±3.7 | 15.03±1.1*# | 4.15±0.3# |
| Fibrin | 8.11±0.9 | 6.09±0.3 | 5.21±0.3# | 9.15±0.9 | 4.25±0.8*# | 4.08±0.5 |
| Leukocytes | 22.17±1.7 | 18.75±3.5 | 9.73±0.5# | 21.95±3.9 | 17.21±2.5 | 8.92±0.8# |
| Lymphocytes | 11.01±0.9 | 14.15±0.8 | 20.12±7.3# | 10.27±0.9 | 15.95±1.9 | 19.75±1.3 |
| Fibroblasts | 6.88±0.5 | 10.05±1.3 | 18.36±3.5# | 7.07±0.8 | 10.21±0.7 | 21.03±1.5# |
| Vessels | 17.23±2.5 | 10.80±2.5# | 9.91±0.8# | 13.14±1.5 | 8.2±0.9*# | 4.84±0.3*# |

Note: * – reliability of differences in comparison with the NCt group ($p < 0.05$);
# – reliability of differences in comparison with the previous period ($p < 0.05$).

**Table 2 – Morphometric indices of the composition of adhesions in rats with chronic pleural empyema against the background of the administration of platelet-rich plasma to potentiate adhesiogenesis (M±m, %)**

| Indices, VF (%) | Experimental groups | | | | | |
|---|---|---|---|---|---|---|
| | NCpe | | | PRPpe | | |
| | Day 10 | Day 20 | Day 30 | Day 10 | Day 20 | Day 30 |
| Collagen fibers | 1.09±0.7 | 11.13±1.7# | 30.08±3.1# | 8.19±0.8* | 23.07±1.5*# | 32.64±4.1# |
| Reticular fiber | 33.72±2.3 | 29.16±3.6# | 7.26±0.8# | 30.75±2.9 | 15.28±3.5*# | 5.11±0.5*# |
| Fibrin | 9.23±0.8 | 7.95±0.9 | 5.83±0.3# | 10.67±0.7 | 8.17±0.9 | 4.97±0.1# |
| Leukocytes | 21.16±1.3 | 19.31±2.1 | 11.12±1.1# | 23.13±4.1 | 16.81±3.3 | 8.13±0.9 |
| Lymphocytes | 13.11±1.9 | 14.29±0.7 | 19.19±5.7 | 12.87±0.8 | 16.03±1.1# | 21.40±1.1 |
| Fibroblasts | 8.02±0.3 | 9.83±1.1 | 17.23±2.9 | 6.92±0.8 | 12.91±0.8 | 19.73±1.1 |
| Vessels | 13.67±1.9 | 8.33±0.3 | 9.29±0.9 | 7.47±1.1# | 7.73±0.9*# | 8.02±0.5 |

Note: * – reliability of differences in comparison with the NCpe group ($p<0.05$);
# – reliability of differences in comparison with the previous period ($p<0.05$).



**A** **B** **C**

**Figure 8 – Pleural cavity of experimental pleural empyema in the comparison group on day 10**

Note: A. Negative control group (NCpe). The cavity is filled with liquid pus, multiple abscesses. B. Comparison group (CGpe). C. Experimental group (PRPpe).

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When determining the VF of collagen and reticular fibers forming adhesions, significant differences were found in the NCt group and in the PRPt experimental group. Thus, on the 10th day of the experiment in the NCt group, the VF of collagen fibers was significantly less than in the experimental group with stimulation of adhesiogenesis with platelet-rich plasma (3.04±0.8% and 8.23±0.9%, respectively) p<0.05. Against the background of an increase in the proportion of collagen fibers, there was a significant decrease in the VF of reticular fibers, which sharply decreased on the 30th day of the experiment (p<0.05).

Besides, at all stages of the experiment, significant differences were detected in the NC group and experimental groups in determining the VF of the cellular composition of adhesions: leukocytes, lymphocytes, fibroblasts (p<0.05).

Thus, as a result of a complex morphological study, the following was found out: biological potentiation of adhesiogenesis by plasma enriched with platelets is an effective measure for stabilizing the rib cage in chest injuries with multiple rib fractures.

### Efficieny of plasma, platelet-enriched through intrapleural administration for potentiating adhesiogenesis in chronic pleural empyema, according to the results of macroscopic examination

As a result of the study, significant differences were established in the morphogenesis of residual cavities in animals of the comparison groups and experimental groups. When conducting a comparative characteristic of experimental chronic pleural empyema and adhesions in the pleural cavity without treatment and with various methods of biological stimulation of adhesion formation, the following results took place: on the 10th day of the experiment, in all the study groups including the negative control group, the comparison group and the three experimental groups, the residual cavity was preserved macroscopically. The severity of the adhesive process depended on the tactics of managing the residual pleural cavity, which arose during experimental modeling of pleural empyema.

In the NCpe group, the volume of the residual pleural cavity was maximum and averaged 25.1±3.1 $mm^3$. Basically, single adhesions were determined in the animals of this group (50.0%); adhesions were absent in 36.7% of cases, multiple adhesions were found only in 13.3%, while spider adhesions were determined predominantly morphologically. The cavity was filled with liquid pus with no signs of organization. The visceral pleura was thickened to 1-1.5 mm, abscesses with a diameter of 1 to 3 mm were determined in its thickness (Fig. 8A).

In the CGpe, as well as in the NCpe group, the total obliteration of the residual cavity was not observed. However, intrapleural adhesions, both single (in 56.7%) and multiple (in 23.3%), were determined. The volume of the residual pleural cavity was less than in the NC group – 23.2±2.5 $mm^3$ (p>0.05).

In the CG, the residual empyema cavity was formed by the parietal pleura, visceral pleura of the upper lobe, an interlobar groove, a lower lobe and a diaphragm. In the thickness of the pleura, multiple encapsulated abscesses up to 3 mm in diameter were determined. There were focal fibrin deposits on the pleural surface (Fig. 8B).

In the experimental group, on the 10th day of experimental PE, against the background of the administration of platelet-enriched plasma, residual cavities of a small volume were determined, fragmented into sectors by single organ adhesions. Intrapleural adhesions were most often located in the costophrenic sinus; they were penetrated by newly formed vessels, which indicated the active formation of adhesions (Fig. 8C).

On the 20th day of experimental EP in the NC group, a residual cavity containing purulent exudate, was determined. The pyogenic membrane was thickened due to the deposition of fibrin up to 2 mm, infiltrated by numerous microabscesses. The dome of the diaphragm was smoothed, the sinus deformity was detected, while there were no intrapleural adhesions (Fig. 9A).

In the antero-inferior parts of the pleural cavity in CGpe rats, a residual empyema cavity with thickened walls, containing organized exudate, was determined. Numerous adhesions, mainly membranous and planar, were localized in the pleurophrenic sinuses and the interlobar sulcus. A pronounced intra-adhesion inflammatory process and rich neovascularization indicated the active formation of adhesions (Fig. 9B).

Residual cavities without purulent contents, with focal fibrin deposits on the walls, up to 2 mm thick, were determined deformed (due to the adhesion process) during biological potentiation of adhesiogenesis as a result of the administration of platelet-rich plasma. Morphologically, adhesions were represented by a wide spectrum: single organ adhesions were combined with multiple filmy and ribbon-like adhesions, which were randomly located inside the residual cavity, significantly reducing its volume (Fig. 9C).

By the end of the experiment (day 30), the cases of elimination of residual cavities had been recorded in all the groups. However, in the NCpe group, the empyema cavity was determined significantly more often than in the comparison group and in the experimental PRPpe group (p<0.05). At the same time, in the NCpe group, the volume of the residual cavity was the largest (19.3±1.7 $mm^3$). There was thickening of the parietal and visceral pleura, with subpleural abscesses. In the animals of this group, single adhesions prevailed (50.0%), in 30.0% of cases adhesions were absent, multiple adhesions were found only in 13.3%, the total obliteration was recorded only in 6.6% (Fig. 10A).

When doxycycline was administrated (CGpe), the volume of the residual empyema cavity averaged 16.5±1.5 mm3, the residual cavity was found to be obliterated in 13.3%; in 16.7% there were single adhesions, in 46.7% – multiple ones. Adhesions were represented by mature massive moorings located in the lower sections (Fig. 10B).

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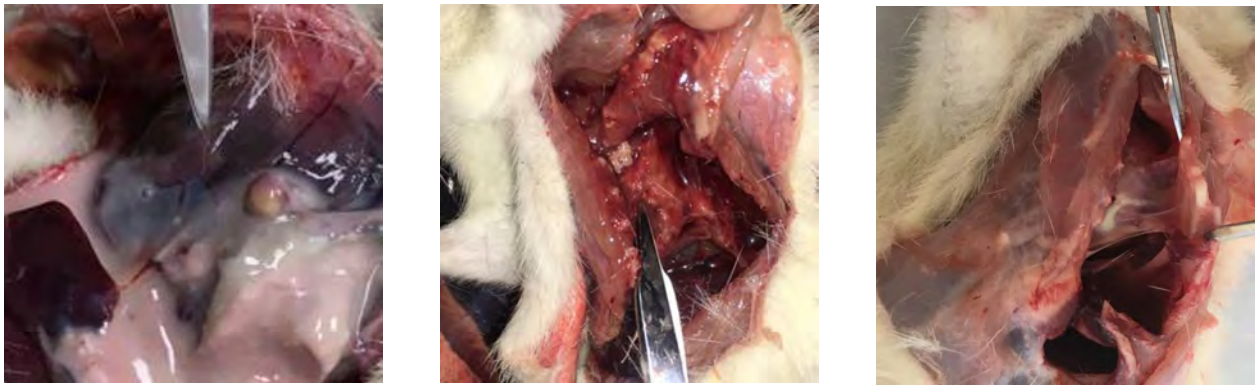
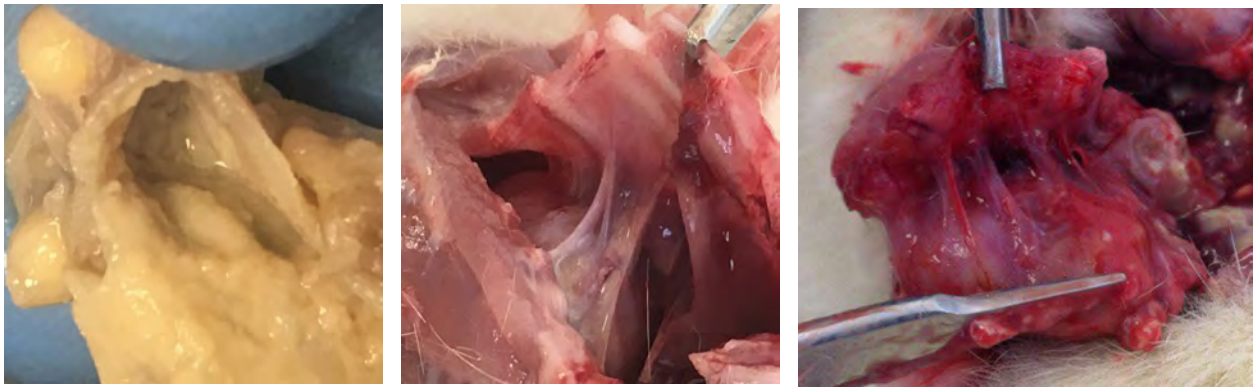


**A** **B** **C**

**Figure 9 – Pleural cavity of experimental pleural empyema on day 20**

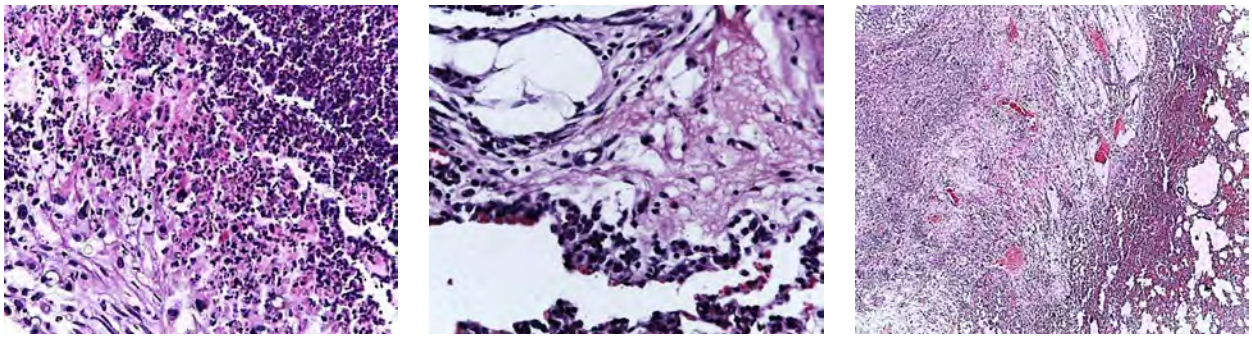
Note: A. Negative control group (NCpe). The cavity is filled with liquid pus, multiple abscesses. B. Comparison group (CGpe). C. Experimental group (PRPpe).



**A** **B** **C**

**Figure 10 – Pleural cavity on the 30th day of experimental pleural empyema**

Note: A. Negative control group (NCpe). B. Comparison group (CGpe). C. Experimental group (PRPpe).



**A** **B** **C**

**Figure 11 – Formation of immature connective tissue in pleural empyema on day 10 of the experiment against the background of chronic pleural empyema in rats**

Note: A. Negative control group (NCpe). B. Comparison group (CGpe). C. Experimental group (PRPpe) with the administration of platelet-rich plasma. Staining with hematoxylin and eosin. Magnification ×400.

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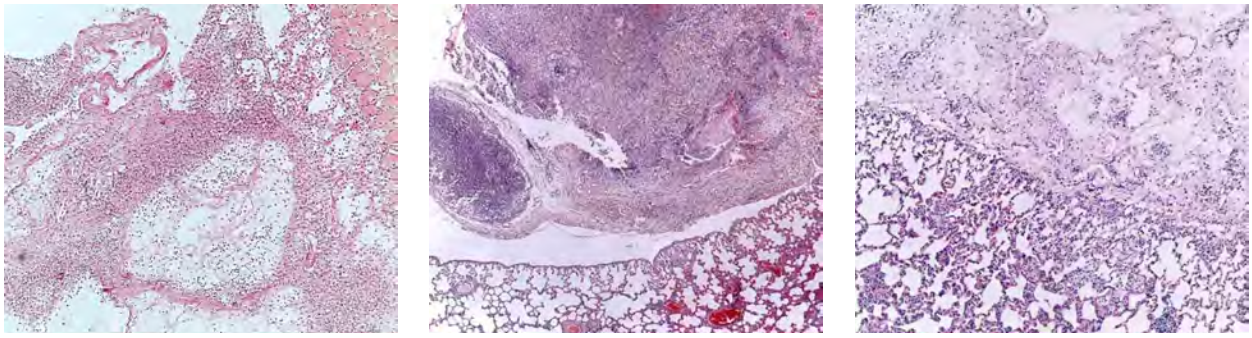
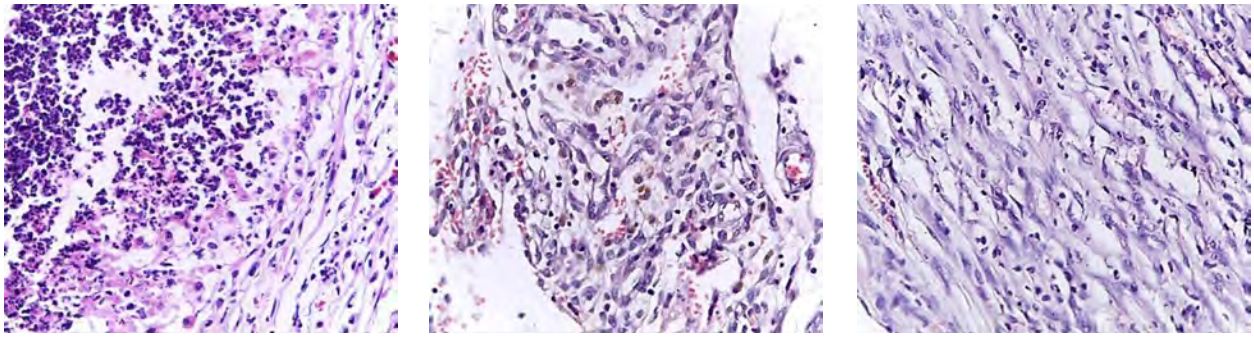


A B C

**Figure 12 – Experimental pleural empyema on day 20 of the experiment**

Note: A. NCpe group. Staining with hematoxylin and eosin. Magnification x100. B. Comparison group. Staining with hematoxylin and eosin. Magnification ×100. C. Experimental group (PRPpe) with the administration of platelet-rich plasma. The phase of young adhesions with the presence of immature connective tissue against the background of chronic pleural empyema in rats on day 20 with the administration of platelet-rich plasma. Staining with hematoxylin and eosin. Magnification ×100.



A B C

**Figure 13 – Experimental pleural empyema on day 30 of the experiment**

Note: A. NCpe group. B. Comparison group. C. Experimental group (PRPpe) with the administration of platelet-rich plasma. Mature adhesion in the pleural cavity of rats with the presence of oriented connective tissue fibers, a small number of lymphocytes against the background of chronic pleural empyema. Staining with hematoxylin and eosin. Magnification ×400.

In the experimental group (PRPpe) on the 30th day, the total obliteration of the empyema cavity was registered in 13.3%. The volume of the residual pleural cavity averaged 12.1±0.8 mm$^3$. The absence of adhesions was registered in 20.0%. Ribbon-like and planar adhesions were morphologically identified (Figure 10C).

### Efficiency of plasma, platelet-enriched through intrapleural administration for potentiating adhesiogenesis in chest trauma, according to the results of histological study

On the 10th day of the experiment in the pleural cavity in the NCpe group, the impregnation of the pleural layers with purulent fibrinous exudate was determined; the involvement of adjacent tissues in the pathological process was detected. Deposits of fibrin filaments were determined in the inflammatory detritus of the exudate. In the parietal pleura, reactive changes, characterized by the phenomena of nuclear hyperchromia and violations of the nuclear-cytoplasmic relationship, were found out. The inflammatory infiltrate was represented by an abundance of polymorphonuclear neutrophils with single lymphocytes and macrophages (Fig. 11A).

In the comparison group, purulent-fibrinous exudate was also found in the residual PE cavity. The signs of inflammation were revealed, consisting in visualization of mesothelial cells with inflammatory changes. In pleural adhesions, chaotically located connective tissue fibers, mainly reticular, were determined, in the thickness of which there were single fibroblasts and multiple neutrophilic leukocytes (Fig. 11B).

In the experimental group, the residual pleural cavity was delimited from the lung tissue due to the formation of granulation tissue represented by connective tissue fibers with an abundance of blood vessels (Fig. 11C).

In the NCpe group, on the 20th day of experimental EP, a partial organization of exudate with a precipitate of fibrin and single connective tissue fibers was revealed. A few microabscesses with a tissue dendrite located in the center, were found. The pathological process was delimited by immature connective tissue fibers, inside which there was a massive inflammatory infiltration, represented by neutrophils with single lymphocytes and macrophages. There was a discrepancy between the degree of maturity of pleural adhesions and the timing of the experiment (Fig. 12A).

When doxycycline was administrated on the 20th day of the experiment, adhesiogenesis had a number of features associated with fragmentation of the residual cavity by connective tissue adhesions into microcavities containing an insignificant amount of serous-purulent exudate. The detected adhesions were infiltrated with neutrophilic leukocytes and richly vascularized, which indicated active neoangiogenesis (Fig. 12B).

The administration of platelet-rich plasma at this time of the experiment led to the active formation of connective tissue, represented by a network of loosely located thin fibers with edema phenomena. At the same time, the severity of the inflammatory response was significantly lower than in the NCpe and CGpe groups. Morphologically, moderate diffuse lymphocytic infiltration of pleural adhesions with single neutrophilic leukocytes was determined. On the part of the microvasculature, the signs of active neoangiogenesis were revealed, the vessels were evenly distributed among the fibers of the connective tissue (Fig. 12C).

Histological examination of pleural tissues in the negative control group on the 30th day showed a pronounced edema of the connective tissue, abundantly infiltrated by neutrophils, single plasmocytes and macrophages. The mesothelium of the visceral and parietal sheets was not visualized, between the thin bands of the connective tissue exudate was determined; its composition was mainly represented by neutrophils. Diffuse impregnation and thickening of the pleural sheets due to the pronounced edema and abundant neutrophilic infiltration was revealed. The vessels of the microvasculature had moderate blood filling, with symptoms of perivascular edema, which was histologically manifested by the fragmentation of the vascular wall and the presence of optically empty perivascular spaces (Fig. 13A).

Against the background of doxycycline administration, in the animals of the comparison group the presence of single immature adhesions in the pleural cavity was detected. The adhesions were formed by loose fibrous connective tissue, while thin connective tissue fibers had a different direction, between which an abundance of moderately plethorical vessels was revealed. Between the fibers of the connective tissue, an accumulation of fibroblasts was clearly visible; they had rounded nuclei and a small amount of cytoplasm. In addition, lymphocytic infiltration with the presence of single plasma cells was determined. In pleural adhesions, hemosiderin granules as well as large cells with the presence of brown pigment in the cytoplasm (siderophages) were determined. Attention was drawn to the fact that the vessels of the microvasculature were lined with endothelial cells with a rounded nucleus, which indicated "irritation" of the endothelium and was a morphological sign of endothelial dysfunction (Fig. 13B).

In the experimental group with biological potentiation of adhesiogenesis in the pleural cavity with plasma enriched with platelets, the formation of multiple adhesions with the presence of strictly oriented connective tissue fibers was registered; fibroblasts and fibrocytes with an elongated nucleus and a small amount of cytoplasm were detected there. Single vessels with typical endothelium were visualized. Morphological signs of inflammation were minimal and characterized by the presence of single lymphocytes and plasma cells (Fig. 13C).

### Morphometric essessment of the efficiency of platelet-rich plasma for potentiation of adhesion in chronic pleural empyema in dynamics

The summary data on the results of the morphometric study in the animals with the experimental chest trauma of the NCpe group and with various methods of biological stimulation of adhesiogenesis, are presented in Table 2.

As a result of a complex morphological study, it was found out that the composition of the adhesions within the experimental groups varied significantly. Thus, during the entire observation, the VF of collagen fibers forming adhesions was higher in the group with biological stimulation of adhesiogenesis than in the NCpe group and in the CGpe group. On the 10th day, in the group with the use of PRP technologies, this indicator was 7.5 times higher than in the group of NC ($p<0.01$). As the duration of the experiment increased, the VF of collagen fibers in the adhesions formed in the NC and CG group steadily increased. On the 30th day it did not have significant differences from the adhesions softened during potentiation of adhesiogenesis.

Alongside this with an increase in the VF of collagen fibers, a decrease in VF of reticular fibers occurred, which indicated the maturation of the adhesion. However, in the PRP group, this indicator, already at the initial stages of the experiment, was significantly lower than in the NC and CG groups ($p<0.05$). The changes in the cellular composition of adhesions were less pronounced, but leukocyte infiltration significantly decreased in the PRPpe group compared to the NC and CG groups. So, on the 20th day, in the group with combined stimulation of adhesions with the plasma enriched with platelets, the VF of leukocytes was 2.1 times lower than in the NC group. Alongside with this, there was an increase in VF of lymphocytes and VF of fibroblasts, which was recorded significantly earlier (10-20 days) than in the NC group.

Thus, the results obtained indicate the earlier formation and maturation of adhesions during the biological stimulation of platelet-rich plasma, as well as of their stability in this group.

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## DISCUSSION OF RESULTS

Adhesiogenesis is a compensatory reaction that occurs in response to surgical (or any other) trauma. Platelet-rich plasma (PRP technologies) was chosen as a biological substance that provides potentiation of adhesiogenesis.

The choice of a chest injury with multiple floating fractures of ribs and residual cavity in chronic pleural empyema as nosological units for the use of biotechnology in stimulation of adhesion is not accidental. In chest trauma, stimulation of the adhesions should have a dual role: stabilization of the rib cage – on the one hand and protection of the lung parenchyma from damage – on the other. In pleural empyema, the residual cavity is invaded with connective tissue, which leads to its obliteration and elimination of the chronic focus of purulent infection.

According to the literature data, the use of platelet-rich plasma is pathogenetically justified, because platelets contain growth factors (PDGF, VEGF, EGF, FGF, etc.) that increase the activity of fibroblasts. Fibroblasts produce elastin, collagen, hyaluronic acid, promoting the formation of connective tissue and its neovascularization. In addition, the growth factors inhibit the decrease in bone tissue volume by stimulating the proliferation of osteoblasts and blocking osteoclasts. There is some information about the immunostimulating effect of PRP, its participation in the normalization of metabolic processes, tissue respiration, optimization of microcirculation [15, 16].

For chest trauma, a model for stabilizing the rib cage by stimulating adhesiogenesis using platelet-rich plasma has been developed. This model is pathogenetically substantiated, has validity and makes it possible to assess the morphological structure of adhesions formed under the influence of platelet growth factors. When modeling and treating experimental chest trauma with multiple rib fractures, significant differences were found out in the negative control group and in the experimental group. When assessing the severity of the adhesion process, it was found out that adhesions are most often visualized at the sites of rib fractures (from 13.3 to 40%). At the same time, in the NC group, single adhesions prevailed (23.3%-63.3%), while in the experimental group on the 20$^{th}$ and 30th days, single adhesions were absent ($p<0.01$). Similar results were obtained when analyzing the cases of the absence of adhesions: the animals without adhesions in the pleural cavity were determined in the NC group at all periods of the experiment (16.7% on the 10$^{th}$ and 20$^{th}$ days; 13.3% on the 30$^{th}$ day). While in the group with PRP technology, the percentage of rats without adhesions in the pleural cavity was significantly lower: from 6.7% on day 10, 3.3% on day 20, to 0% on day 30 ($p<0.05$).

The macroscopic differences in the structure of adhesions are noteworthy; arachnoid and membranous adhesions predominated in the NC group, while ribbon-like and planar adhesions prevailed in the experimental groups. The thickness and density of adhesions was increasing in the duration of the experiment in all the studied groups.

So, this study makes it possible to conclude that the biological potentiation of adhesion is a logical measure of stabilization in chest injuries with multiple rib fractures. The morphological substrate of this method is the formation of mature adhesions without manifestations of activity and further development, thereby confirming the maturity and formation of adhesions. The results obtained are consistent with the literature data, according to which the use of PRP technology has a stimulating effect on the development and neovascularization of adhesions due to the contained growth factors; in addition, there is information about the stimulating effect of PRP technology on the formation of callus [17].

When modeling and treating experimental pleural empyema, the authors' methods of biological stimulation of adhesiogenesis by injecting platelet-rich plasma was used. The elimination of the residual pleural cavity is based on the stimulation of adhesion formation by the growth factors contained in PRP, which is consistent with the literature data [18, 19].

At the same time, significant differences were established in the morphogenesis of residual cavities in the animals of the NC group, comparison groups and the experimental group. It is noteworthy that in the comparison group (treatment with doxycycline) the adhesions consisted mainly of loose fibrous connective tissue infiltrated with cellular elements, which confirmed the fact of the inflammatory process in the adhesion and is prognostically unfavorable in terms of the occurrence of complete obliteration of the pleural cavity adhesions. At the same time, in the experimental group, intrapleural adhesions were formed mainly by connective tissue fibers containing lymphocytes, histiocytes, fibroblasts, a small number of desolate vessels, and no signs of inflammation were detected. The described histological picture is typical of mature adhesions. It means that with targeted administration of PRP into the pleural cavity, adhesiogenesis can be considered controlled.

So, in the experiment it was established that the applied biotechnologies make it possible to potentiate intrapleural adhesions in residual cavities in chronic pleural empyema, which leads up to the complete obliteration of the empyema cavity – a chronic source of infection and a risk factor for disease recurrence.

## CONCLUSION

As a result of the experimental study, the efficiency of the use of platelet-rich plasma for the biological potentiation of adhesiogenesis in experimental chest injuries and chronic pleural empyema has been proved. The results obtained may be a sufficient basis for recommending clinical trials.

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## AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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