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THE STUDY OF THE EFFECTS OF COLD HELIUM PLASMA IN AN EXPERIMENT WHEN MODELING CONTAMINATED AND PURULENT WOUNDS IN RATS

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Summary. A wound (Latin vulnus) is a violation of the anatomical integrity of the integumentary or internal tissues throughout their entire thickness, caused by mechanical action. At all stages of human development, the problem of wound healing has been and remains relevant. In recent years, there has been a noticeable increase in the number of injuries to the head, neck, limbs as a result of military actions, man-made disasters, domestic injuries, road accidents, animal bites. The purpose of the study is to present the experience of using cold helium plasma on experimental models of contaminated and purulent wounds in rats. During the experiment, 2 study groups were formed - group 1 (n = 30) - contaminated wounds; group 2 (n = 30) - purulent. Animals of groups 1 and 2 were divided into 2 subgroups: group 1A (n = 15), which underwent cold plasma sessions, group 1B (n = 15), healing occurred without plasma therapy. Group 2 was divided similarly to the first. A contaminated wound, after shaving the animal's withers, was formed with a sterile scalpel using a 1.5 cm long cut in the skin, subcutaneous fat and muscle layers in the interscapular region. Purulent wounds were modeled according to the method of Sychennikov A.I. Animals of groups 1A and 2A were treated with cold helium plasma of atmospheric pressure for 3 minutes in a course of 9-10 days. When assessing vascular blood flow (SCM-101) in all groups of animals on days 7, 14, 21, it was noted that a high level of blood flow was found in contaminated wounds under the influence of cold helium plasma, allowing for more active restoration of the vascular link. Morphological examination revealed that cold plasma has a beneficial effect on the healing of wound skin defects, helping to accelerate regeneration processes with the formation of a soft, elastic scar.

Keywords: *wounds, experimental modeling, cold helium plasma.*

Relevance. The history of wound treatment has been known since ancient times and remains relevant at the present stage [1].

Even prehistoric humans sought ways to heal injuries sustained while hunting and during military clashes. Some of the earliest information can be found on ancient Sumerian clay tablets, dating back to approximately 2500 BC. The texts describe washing wounds with water and milk, followed by the application of a bandage containing honey and resin. From the Smith and Ebers papyri (1600-1500 BC), discovered in the 19th century, it is known that in Ancient Egypt, honey, oil, and wine were used to treat wounds, and wounds were even closed with sutures or adhesives [2].

In recent years, there has been a noticeable increase in the number of wounds to the head, neck, and extremities as a result of military action, man-made disasters, domestic injuries, road traffic accidents, and animal bites [3].

In case of non-surgical injuries, wounds are divided into contaminated, in which there are bacteria in the defect, but there is no suppuration, and infected, with signs of an infectious and inflammatory process [4].

When examining patients with injuries, clinical and instrumental diagnostic methods are used to confirm or clarify the diagnosis [5].

The result of wound treatment should be as close as possible to the complete restoration of the functions of damaged organs and body parts, which affects the satisfactory quality of life of the patient [6].

This is consistent with the WHO concept of continuous improvement of the quality of health care [7].

In the treatment of wounds, some authors recommend the use of hyperbaric oxygenation, regenerative dressings, and cold plasma exposure [8].

At the present stage, plasma therapy is actively used in cosmetology, namely cold helium plasma, which has high antibacterial, antioxidant and regenerative properties.

The aim of the study was to present the effect of cold plasma on the quality of scar formation using experimental modeling of contaminated and purulent wounds on animals.

Materials and methods. At the Department of Experimental Medicine with a vivarium of the Privolzhsky Research Medical University, a study of contaminated and purulent wounds was performed on 60 sexually mature male Wistar rats weighing 150-200 g, obtained from the Andreyevka branch of the Federal State Budgetary Scientific Institution "Scientific Center for Biomedical Technologies". All animals were kept under standard vivarium conditions in cages with free access to food and water. The working conditions complied with the principles of biological ethics, the requirements of the International Helsinki Convention for the Humane Treatment of Animals (1972), the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes ET/S 129 (Strasbourg, March 18, 1986) and the order of the Ministry of Health of the Russian Federation dated April 1, 2016 No. 199n "On approval of the Rules of Good Laboratory Practice". Minutes of the meeting of the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education "Primary Medical University" of the Ministry of Health of the Russian Federation No. 5 dated May 17, 2024.

All animals, after preliminary anesthesia with Zolazepam (25 mg/kg) and Xylazine hydrochloride (10 mg/kg), administered intramuscularly, underwent modeling of deep wounds in the interscapular region with opening of the skin, subcutaneous fat and muscle layers.

During the experiment, two study groups were formed: Group 1 (n=30) – contaminated wounds; Group 2 (n=30) – purulent wounds. Animals from Groups 1 and 2 were divided into two subgroups: Group 1A (n=15), which underwent cold plasma sessions; Group 1B (n=15), which healed without plasma therapy. Group 2 was divided similarly to the first.

A contaminated wound, after shaving the animal's withers, was formed with a sterile scalpel using a 1.5 cm long incision in the skin, subcutaneous fat and muscle layers in the interscapular region.

Purulent wounds were modeled according to the method of Sychennikov A.I.: the hair in the withers area of the animal was shaved, a linear incision of the skin, subcutaneous fat, fascia and muscle of 1.0 cm in length was made with a sterile scalpel. After the incision was made, the walls and bottom of the wound were spread apart with a Kocher clamp. In animals of groups 2A and B, a gauze swab abundantly moistened with a suspension of Staphylococcus aureus and Pseudomonas aeruginosa cultures with a concentration of microbial bodies of 109 per ml of isotonic sodium chloride solution (identical strains of microorganisms were used in all cases) was placed into the wound. After this, an adaptive suture was applied to the skin with 1-0 thread. The swab was exposed for 5 days. During this period, a purulent process formed in the wounds [9].

Animals of groups 1A and 2A were treated with cold helium plasma at atmospheric pressure for 3 minutes every 24 hours for a course of 9-10 days [10-13] (Figure 1).

Methodology. The cold plasma generator consists of a power source generating voltage pulses with an amplitude of 150 V and a repetition rate of 17 kHz, and a cylindrical applicator increasing the voltage to a breakdown level of 10 kV. High voltage is applied to two ring electrodes encircling a quartz tube purged with an inert gas flow at a rate of 2 l/min. Highly purified helium (99.99%) was used to eliminate the toxic effects of foreign gas impurities and facilitate electrical breakdown. The ground electrode was located closer to the edge of the applicator downstream of the flow. A barrier dielectric discharge through the tube walls generated a flow of cold, weakly ionized plasma with a charged particle concentration of approximately 10 cm-3. The term "cold" means that the jet temperature does not exceed 40 degrees Celsius, and the plasma has no thermal effect on living tissue. Temperature changes during plasma treatment did not exceed the statistical measurement error. Temperature in the treatment area was monitored remotely using a Fluke 59 max pyrometer. The plasma jet length was 2.5-3 cm, with a diameter of 2 mm (Figure 2).

During the treatment and observation process, the state of skin blood flow in all groups of rats was assessed using the skin electrometry technique, which has previously demonstrated high diagnostic information value in both experimental and clinical studies.

For this purpose, a device was used – the SCM-101 skin conductivity meter..

On the 21st day from wound formation, under general anesthesia, scar excision was performed for morphological examination. Animals were withdrawn from the experiment by decapitation.

The data for this study were processed using Excel and the STADIA statistical package. The "Descriptive Statistics" tables present the basic numerical characteristics of the studied samples: mean (M), standard deviation (σ), error of the mean (m), median, interquartile range, and information

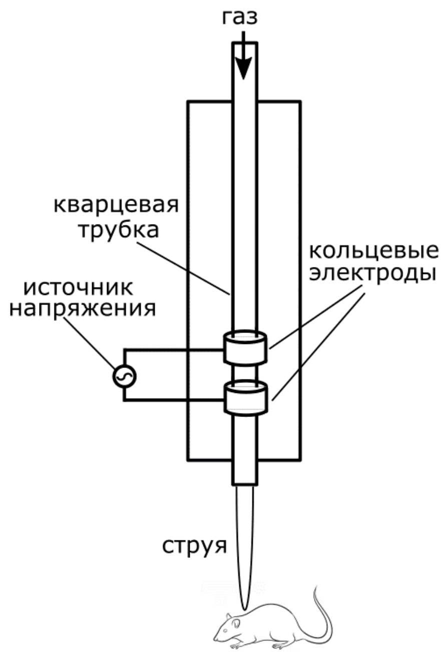


Figure 1. Schematic diagram of a cold plasma source

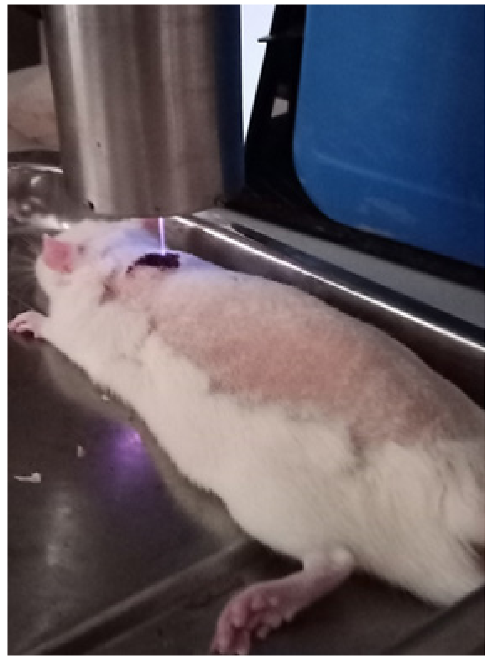


Figure 2. Conducting a cold helium plasma session in an experiment

about the sample distribution (N – nearly normal distribution, ≠N – non-normal distribution). Excel and the STADIA package were used for calculations.

To analyze the distribution of samples for proximity to normality, the following were used: the A and E analysis method (asymmetry and kurtosis), the Kolmogorov method, the ω2 method (omega square), the χ2 method (Pearson method) – from the STADIA package.

The vast majority of samples had distributions different from normal, therefore, at the stage of comparing samples, nonparametric methods were used - Wilcoxon, Wilcoxon-Mann-Whitney, Van der Wald and the sign test, which compare samples by medians.

The comparison tables indicate the samples being compared, the result of the comparison (there is a difference/there is no difference), the significance level p at which the Ho-hypothesis was tested, and the name of the numerical characteristic with which the Ho-hypothesis was tested (Ho: no difference in medians).

Table 1. Descriptive statistics of contaminated and purulent wounds

	Группы	Обознач. выборки	Название выборки	Среднее M	□□-□â. î □ëî í . σ	Ошибка выб.ср.m	Медиана Me (II квартиль)	Межкварт. размах (I - III)	Распре-деление
Кожный кровоток рана 1	7 день	1	рана	34,60	49,85	12,87	5	4 67	≠N
		2	контроль	32,00	33,05	8,53	24	9 44	≠N
	14 день	3	рана	38,60	42,71	11,03	18	6 63	≠N
		4	контроль	18,27	17,79	4,59	7	2 39	≠N
	21 день	5	рана	41,67	21,40	5,53	44	27 54	N
		6	контроль	26,93	40,50	10,46	9	6 21	≠N
Кожный кровоток рана 2	7 день	7	рана	54,80	52,61	13,59	48	10 79	≠N
		8	контроль	40,07	30,13	7,78	36	13 76	≠N
	14 день	9	рана	30,20	26,46	6,83	24	5 54	≠N
		10	контроль	31,47	38,73	10,00	18	5 38	≠N
	21 день	11	рана	18,87	16,06	4,15	13	5 31	≠N
		12	контроль	27,53	24,62	6,36	21	10 35	≠N

Table 2. Comparisons in groups 1A, 2A

Признаки	Сравниваемые выборки	Результат сравнения: различие	Ó□î â□í ü çí □÷. □	Различие
рана 1 - рана 2 (7 день)	**1 ~ 7**	**есть**	**<0,05**	в медианах
рана 1 - рана 2 (14 день)	**3 ~ 9**	**нет**	**>0,05**	в медианах
рана 1 - рана 2 (21 день)	**5 ~ 11**	**есть**	**<0,01**	в медианах
Сравнение со своим контролем				
рана 1	**1 ~ 2**	**нет**	**>0,05**	в медианах
	3 ~ 4	**есть**	**<0,05**	в медианах
	5~ 6	**есть**	**<0,05**	в медианах
рана 2	**7 ~ 8**	**нет**	**>0,05**	в медианах
	9 ~ 10	**нет**	**>0,05**	в медианах
	11 ~ 12	**есть**	**<0,05**	в медианах
Динамика				
рана 1	**1 ~ 3**	**нет**	**>0,05**	в медианах
	3 ~ 5	**нет**	**>0,05**	в медианах
рана 2	**7 ~ 9**	**есть**	**<0,05**	в медианах
	9 ~ 11	**нет**	**>0,05**	в медианах

Results and discussions. Vascular blood flow was assessed in all animal groups on days 7, 14, and 21. In contaminated wounds, the use of cold plasma throughout the healing period stimulated an increase in cutaneous blood flow compared to the control group (1B), which did not receive plasma therapy.

It was noted that during the inflammation phase (phase I), increased blood flow was recorded in the group of animals with purulent wounds 2A, B (54.80±13.59; 40.07±7.78), which is associated with greater activation of the immune system in the acute period. In animals 2A, B on the 14th day - in the regeneration phase (phase II), when two main processes occur in the wound: collagenization and intensive growth of blood and lymphatic vessels, this indicator decreased to 30.20±6.83 in group 2A, and in group 1A - increased to 38.60±11.03.

In the phase of scar formation and reorganization on day 21 (phase III), the blood flow level was high (41.67±5.53) in group 1A; in animals of group 2A, a noticeable decrease in blood supply at the edges of the wound was observed (18.87±4.15) (Table 1, 2).

Thus, by analyzing the level of blood flow restoration in different phases of wound healing, it has been proven that in contaminated wounds, under the influence of cold helium plasma, the vascular link is restored more actively, which confirms the high level of blood flow.

Morphological examination of scars in groups 1A and 2A, which underwent plasma therapy, revealed that the epidermis in the damaged area showed signs of epithelial cell proliferation in the spinous and granular layers, indicating active regeneration of the superficial skin layers. The dermis showed abundant lymphohistiocytic infiltration with an admixture of segmented leukocytes. At the base of the defect, granulation tissue proliferation was observed, consisting of thin-walled vessels, inflammatory infiltration, and chaotic fibrous tissue bundles (Figure 3).

A tendency toward rapid epithelialization of the wound defect is also noted. In the dermis, areas of tissue with hemorrhagic impregnation and moderate lymphohistiocytic infiltration with isolated segmented cells are observed. At the base of the defect, a thin layer of granulation tissue with thin-walled vessels, moderate inflammatory infiltration, and mild fibrosis is visible (Figures 4, 5).

In morphological specimens from animals in groups 1B and 2B, repair processes predominated over regeneration in the scar tissue. Thinned epidermis indicated a delayed epithelialization process and, consequently, reduced resistance to external environmental factors. The dermis exhibited disrupted histoarchitecture due to fibrous tissue proliferation, chaotically distributed skin appendages

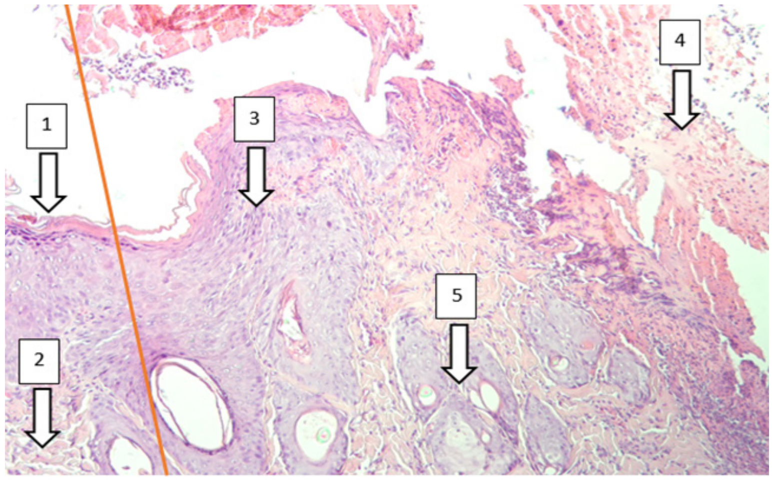


Figure 3. Wound defect zone with an active regeneration process: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrin clot, 5 – skin appendages. Hematoxylin and eosin staining. Magnification 10×10

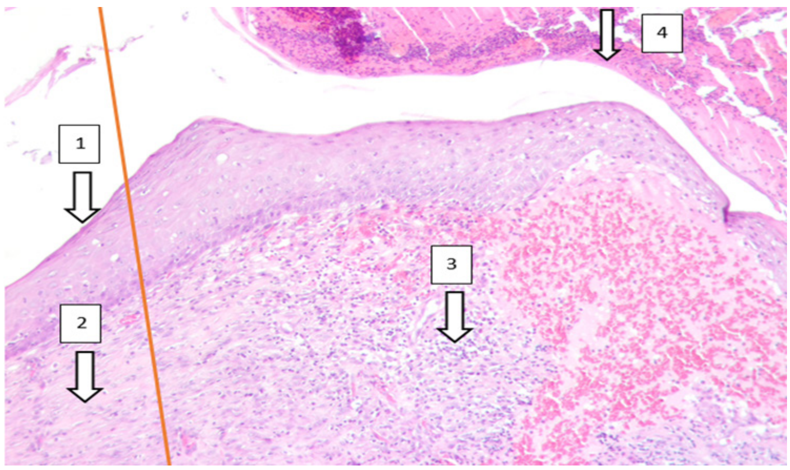


Figure 4. Activation of regeneration processes: lymphohistiocytic infiltration and mild fibrosis. Skin areas with superficial defects: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrin clot. Hematoxylin and eosin staining. Magnification: 10×10

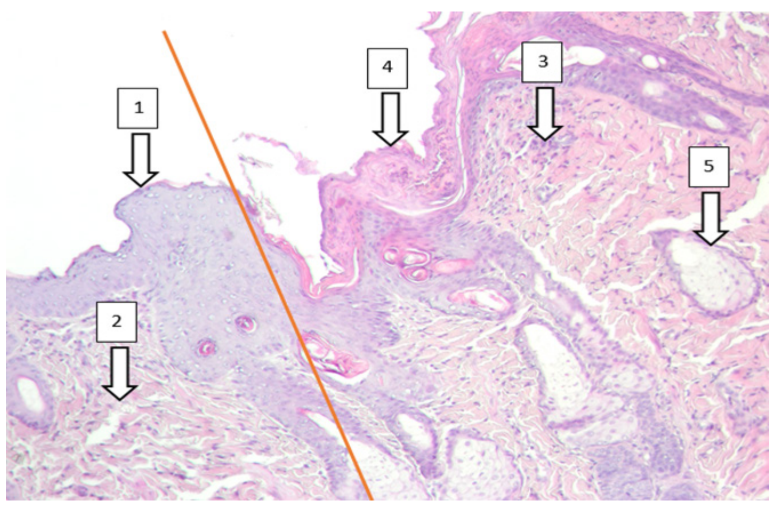
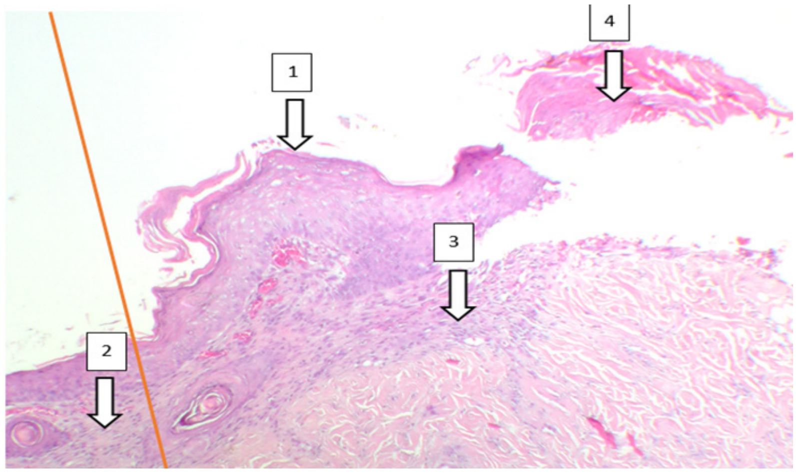
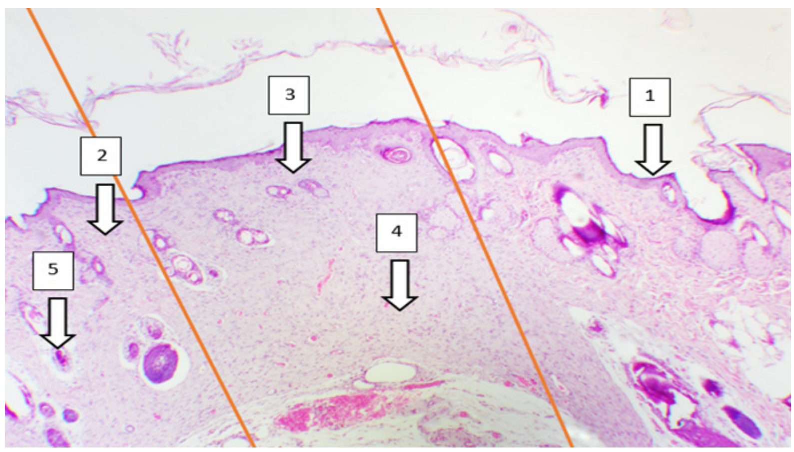


Figure 5. Predominance of regenerative processes with a tendency to form a soft, elastic postoperative scar. Skin areas with superficial defects: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrin clot, 5 – skin appendages. Hematoxylin and eosin staining. Magnification: 10×10



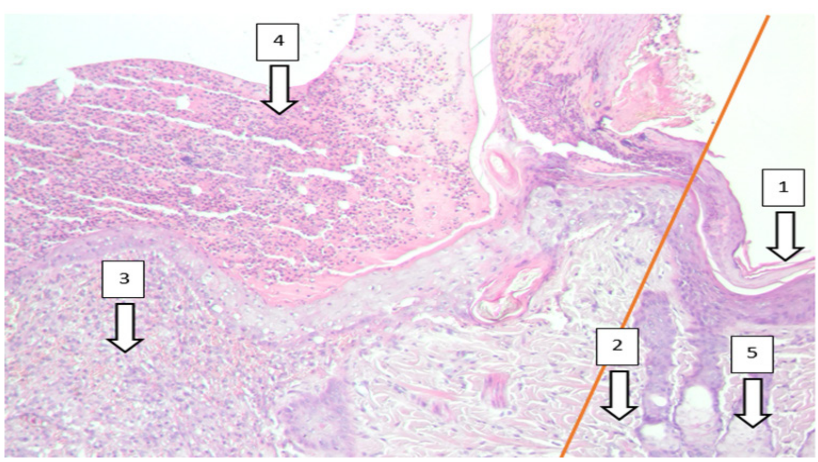
healthy tissues damage zone

Figure 6. Predominance of repair processes over regeneration due to the proliferation of fibrous tissue. Skin areas with superficial defects: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrin clot. Hematoxylin and eosin staining. Magnification: 10×10



healthy tissues “postoperative “scar zone” damage »

Figure 7. Formation of coarse scar tissue with disruption of the histoarchitecture of the skin. Areas of skin with superficial defects: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrosis zone, 5 – skin appendages. Hematoxylin and eosin staining. Magnification: 10×10



“damage zone» «healthy tissues”

Figure 8. Predominance of the alteration process over regeneration. Skin areas with superficial defects: 1 – epidermis, 2 – dermis, 3 – inflammatory infiltration, 4 – fibrin clot, 5 – skin appendages. Hematoxylin and eosin staining. Magnification: 10×10

(hair follicles, sweat glands), and sparse lymphohistiocytic infiltration in the interstitial tissue, indicating an unfavorable postoperative recovery outcome (Figures 5–8).

Thus, the use of cold helium plasma sessions has a beneficial effect on the healing of wound skin defects, promoting active restoration of the vascular link, accelerating the regeneration processes with the formation of a soft, elastic scar.

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