

**AIR PLASMA AS AN EFFECTIVE AND PROMISING METHOD OF TREATMENT OF THE THIRD DEGREE BURNING WOUNDS**

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**ВОЗДУШНАЯ ПЛАЗМА КАК ЭФФЕКТИВНЫЙ И ПЕРСПЕКТИВНЫЙ МЕТОД ЛЕЧЕНИЯ ТУШЕНИЙ ГОРЕНИЯ ТРЕТЬЕГО СТЕПЕНИ**

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**SUMMARY**

The pathophysiological assessment of the treatment results of the third degree skin burns with the use of cold atmospheric plasma in the experiment was performed. During the study, an original technique for reproducing a deep thermal skin burns in small laboratory animals (rats) has been developed. It is found that the use of cold atmospheric plasma not only reduces the incidence of purulent complications, but also contributes to shortening of the skin regeneration time by 20% ( $p < 0.05$ ).

**Key words:** deep thermal skin burns, cold atmospheric plasma, wound coverings, regeneration of the skin

**Introduction.** Currently, in combustiology increasingly used a multidisciplinary approach to the treatment of burns, which is implemented taking into account the pathogenesis of burn disease and its complications. All currently known methods of treating deep skin burns do not allow achieving optimal results, leaving a number of unresolved issues, primarily in terms of choosing a fast and effective method of skin regeneration for such lesions. Nowadays, combustiologic centers applying active surgical tactics, which is based on early necrosectomy followed by auto-thermoplasty of burn wounds. A special importance in the treatment of burns is also given to the prevention of purulent complications of such a wound. One of the possible pathophysiological methods to improve the results of therapy of patients with extensive deep burns is the use of physical influence on the wound surface, in particular, the use of cold atmospheric plasma for this purpose.

The biological effects of cold atmospheric plasma with low-pressure include: antimicrobial and hemostatic, stimulation of tissue regeneration. Its antibacterial effect is caused by damaging the cell wall and membrane of bacteria by ultraviolet radiation and active radicals. In contrast to the existing physical methods to stop bleeding (electrocoagulation, argon-plasma coagulation), cold atmospheric plasma does not damage tissue, but provides hemostasis by accelerating the activation and aggregation of platelets, the formation of a fibrin clot. The issue of the direct effect of cold atmospheric plasma with low-pressure on tissue regeneration in case of damage remains debatable. Some researchers note the acceleration of the proliferation of fibroblasts in vitro when exposed to this type of plasma. There are publications in which the stimulation of the

regeneration of damaged tissues is explained by the combination of antibacterial and hemostatic effects of plasma. Thus, the available data makes the implementation of cold atmospheric plasma with low-pressure for the treatment of burns extremely promising.

The purpose of this research is to study the effect of cold atmospheric plasma on reparative histogenesis in deep skin burns in an experiment.

**Materials and methods.** Experimental research was performed on 40 male outbred rats weighing 230–250 g. All manipulations with animals were performed under general inhalation (ether) anesthesia under aseptic conditions. Reproduction of skin burns of the third degree (ICD-10) was carried out according to our own original developed method (rationalization proposal of the Military Medical Academy No. 14287/1 dated January 19, 2016) (Figure 1).

For the generation of cold atmospheric plasma with low-pressure was used an apparatus manufactured by specialists of the High Voltage, Electrical insulating and Cable technology Department of the Institute of Energy and Transport Systems of St. Petersburg Polytechnic University after Peter the Great (St. Petersburg PU). The device can be held in the hands, and touching the plasma beam to a biological object does not lead to electric shock.

The device is powered by direct current. The output voltage can be changed in the range of up to 20kV. The current source is connected in series with a needle electrode made of stainless steel (tip diameter 50  $\mu\text{m}$ ) through a 120 M $\Omega$  resistor.

When the generator starts, a plasma beam is generated between the tip of the electrode and the biological object, which in its physical essence is similar to a positive corona discharge.



*Figure 1. Rat skin after reproduction of the third degree burn*

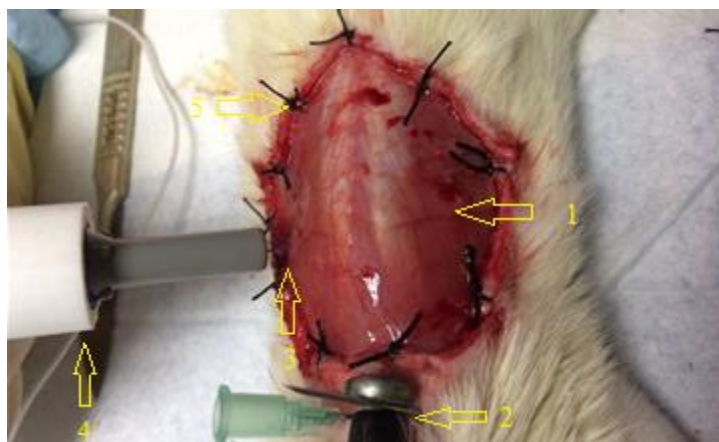
Animals were divided into 3 groups (8 animals in each group) according to the method of treatment.

In the first group of animals, necrotomy (Fig. 2) till own fascia was performed 60 minutes after the third degree burn.



*Figure 2. Wound surface after performing necrotomy*

Immediately after necroectomy, the wound edges were fixed to the underlying tissues with interrupted sutures, then the entire wound surface was treated with cold atmospheric plasma for 10 minutes. The distance between the beam source and the wound surface was 0.5–1 cm (Fig. 4). The device was grounded for the manipulation.



*Figure 3 - The stage of treating of the wound surface with cold atmospheric plasma:  
1 - wound surface; 2 - grounding; 3 - beam of cold atmospheric plasma; 4 - handle of device with insulation;  
5 - single skin-muscular sutures for prevention of wounds contraction*

In the second group, early necrotomy and treatment of wounds of animals was not carried out (control group).

In the third group, without wounds treatment early necrectomy was performed (second control group).

Evaluation of the effectiveness of selected methods of treatment and wounds' photographing was performed every three days. The wounds were examined as well as the character of the discharge, the presence and type of granulations were noted, and the terms of the scab rejection and wound healing were recorded.

With planimetric method by L.N. Popova the area of the wound was determined and the index of healing was calculated by the following formula (Fenchin KI, 1979):

$$\frac{(S - S_n) \times 100}{S_n \times T},$$

where, S – wound area in the previous measurement, mm<sup>2</sup>;

S<sub>n</sub> – wound area at the current measurement, mm<sup>2</sup>;

T – interval between measurements, days

The selection of biopsy specimens for histological examination was carried out on 3rd, 7th, 12th, 15th, 21th, 28th days of the treatment. Biopsy specimens

were fixed in a 10% solution of neutral formalin, followed by posting through alcohols of upward concentration (30% -100%) and pouring into paraffin. Paraffin sections were stained with hematoxylin and eosin with their further study by methods of light microscopy.

Processing of the results was carried out in accordance with generally accepted methods of variation statistics. As the criterion of validity was considered value  $p < 0.05$ .

**Results and discussion.** The regeneration process in the zone of a deep burn of the third degree with the use of cold atmospheric plasma with low-pressure had distinguishing features. On 21<sup>st</sup> day of the research, this group of animals showed a reduction in the area of the wound to 6 cm<sup>2</sup> ( $p < 0.05$ ). Treatment of a burn wound after early necroectomy makes it possible to accelerate regeneration processes in the early postoperative period by 20% ( $p < 0.05$ ), as well as to reduce the area of the scar tissue on the 28<sup>th</sup> day of observation by 52.5% ( $p < 0.05$ ) in comparison to the control group (Table 1).

Performing a radical surgical necrotomy in the zone of deep burn of skin, without further treatment, reduces the wound area by the 21<sup>st</sup> day to 8 cm<sup>2</sup> ( $p < 0.05$ ), the regeneration process is accelerated by 8.6%, after 28 days there is a decrease in the scar tissue area by 10% ( $p < 0.05$ ) compared with the control group.

Table 1

**Planimetric assessment of wounds according to the methods of treatment**

Research groups	Healing time, days	Scar tissue surface, cm <sup>2</sup>
Control group	35 ± 2,9	5 ± 0,1
Cold atmospheric plasma + Necrectomy	28 ± 2,1	3,8 ± 0,4*
Necrectomy without treatment	32 ± 1,6**	4,5 ± 0,8**
* - significant ( $p < 0.05$ ) compared to the animals in the control group		
** – significant ( $p < 0.05$ ) compared to chitosan-with polyamide after necrotomy		

The results of planimetric studies were confirmed by a morphometric estimate of the number of microvasculature vessels in wound biopsy specimens by 35th days of observation. The average number of microvessels in growing granulations against the background of wound treatment with cold atmospheric plasma was 5. Early necrotomy without subsequent treatment allows to increase the number of vessels by 13.4% in comparison to the control group ( $p < 0.05$ ).

In the morphometric assessment of the thickness of the newly formed granulation tissue in the defect

area on the 35th observation day, it was found that by cold atmospheric plasma treatment of wound surface the value of the analyzed parameter is 1145 μm, which is 4.8% more than in the control ( $p < 0.01$ ). Early necrectomy without further treatment enables to increase the thickness of the newly formed granulation tissue in the area of the defect by 1%, compared with the control group. It can be concluded that the use of cold atmospheric plasma contributes to the development of high-grade connective tissue in the zone of deep thermal burn in earlier periods (Table 2).

Table 2.

**The thickness of the newly formed granulations according to the treatment methods**

Study groups	Tissue thickness, micron
Control group (without treatment)	1090,4 ± 25,9
Treatment cold atmospheric plasma	1145,1 ± 44,3 *
Early necrotomy (without treatment)	1100,4 ± 71,7
* – significant ( $p < 0.05$ ) compared to the control;	

**Conclusions and discussion.** The study results indicate that the use of cold atmospheric plasma without further application of wound dressings enables to achieve wound healing by 28 days, i.e. to accelerate the regeneration processes to 20% ( $p < 0.05$ ), and also to

reduce the area of the scar tissue to 52.5% ( $p < 0.05$ ) in comparison to the results of control group animals. Against the background of the implementation of cold atmospheric plasma, an earlier development of mature connective tissue in biopsy specimens is noted, further

the thickness of newly formed granulations exceeds the control to 4.8% ( $p < 0.01$ ). Early necrectomy without further treatment reduces the wound area by 21 days to 8 cm<sup>2</sup>, the regeneration process is accelerated to 8.6% ( $p < 0.05$ ), by 28 days there is a decrease in the scar tissue area to 10% ( $p < 0.05$ ) compared with the control group.

The use of cold atmospheric plasma in the treatment of deep thermal burns of the skin of the third degree (ICD-10) is a promising method. The literature data show that the local application of cold atmospheric low-pressure plasma in the area of the skin defect leads to antimicrobial effect, hemostatic action, and the stimulation of tissue regeneration. These phenomena are due to the generation of free radicals, UV radiation as well as charged particles. The results of our research indicate that the use of plasma in the zone of a deep burn of the third degree enable to reduce the area of the scar tissue to 52.5% ( $p < 0.05$ ).

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### ОСОБЕННОСТИ СВОБОДНО-РАДИКАЛЬНОГО ОКИСЛЕНИЯ И АНТИОКСИДАНТНОЙ ЗАЩИТЫ ПРИ АБДОМИНАЛЬНОМ СЕПСИСЕ В УСЛОВИИ ЭКСТРАКОРПОРАЛЬНОЙ ГЕМОКОРРЕКЦИИ

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#### АННОТАЦИЯ

Целью работы явилось изучение роли оксидативного стресса в патогенезе острого респираторного дистресс-синдрома у больных тяжелым абдоминальным сепсисом и возможности его патогенетической коррекции. Обследовано 46 больных. Все пациенты были разделены на 2 группы. Больным основной группы ( $n=23$ ) наряду со стандартной интенсивной терапией и активной детоксикацией (продленной вено-венозной гемофильтрацией) проводилась метаболическая терапия сукцинатсодержащими антиоксидантами. Больные контрольной группы ( $n=23$ ) получали стандартную интенсивную терапию и активную детоксикацию. Исследование проводили на пяти этапах: до гемофильтрации, а также через 1 сутки, 3, 5 и 7 суток после ее начала. Установлено, что только на 5 этапе изучаемые показатели в основной группе существенно отличались от таковых в контрольной группе, достигая нормальных значений. В контрольной группе существенное улучшение указанных показателей наблюдалось только на пятом этапе. Таким образом, сочетанное применение традиционной терапии тяжелого сепсиса, активной детоксикации и метаболической протекторной терапией сукцинатсодержащими антигипоксантами приводит к более быстрому купированию оксидативного стресса, раннему восстановлению активности системы антиоксидантной защиты и нормализации газообмена в легких.