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COMPARATIVE ASSESMENT OF EFFICIENCY APPLICATION COLD ATMOSPHERIC PLASMA AND BIOPOLYMEROUS COATS EVALUATION OF THE EFFECTIVENESS FOR THE TREATMENT OF SKIN BURNS OF III DEGREE IN EXPERIMENT

© E.V. Zinovev[1], M.S. Asadulaev[1], I.A. Komissarov[1], M.V. Shemet[2], V.E. Yudin[2], R.G. Stoyanovskiy[1], N.V. Smirnova[2], A.S. Shabunin[2], S.A. Lukyanov[1], T.A. Shalonya[1], A.E. Kryukov[2], I.V. Artsimovich[1], D.V. Kostyakov[1]

[1] St. Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Russia;
[2] Peter the Great St. Petersburg Polytechnic University, Saint Petersburg, Russia

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The results of the application of cold atmospheric plasma are discussed in the experimental study. The effectiveness of an experimental wound dressing based on chitosan nanofibers and copolyamide and a commercial wound dressing material based on hyaluronic acid hydrogel in the treatment of third-degree skin burns (*ICD-10*) is also evaluated. During the first phase of the study, an original method of inflicting thermal burn on the skin of small laboratory animals (rodents) was developed. The temperature of dehaired skin and the temperature of skin heated by resistive electrical element metal plate were obtained through a digital thermometer sensor. The source for the generation of cold atmospheric plasma was made by specialists of St. Petersburg Polytechnic University of Peter the Great. Biopsy material for histological assay was taken 3, 7, 12, 15, 21, and 28 days after treatment. Paraffinic microscopic sectioning was performed with hematoxylin and eosin; after this, microscopic assay was done. The use of cold atmospheric plasma decreases the frequency of purulent complications and also helps reduce the recovery time of the skin by 20% ($p < 0.05$) but is not sufficient to achieve the result that has been proclaimed after an early escharectomy and replacement of surgical defects by wound dressing materials based on natural polymers. It was demonstrated that the wound dressing based on aliphatic copolyamide and chitosan and hyaluronic acid hydrogel can help significantly accelerate the process of reparative regeneration and histogenesis in the heat-affected zone after escharectomy for up to 14.6%-46% ($p < 0.05$).

Keywords: deep thermal burns; cold atmospheric plasma; wound coverings; skin regeneration; aliphatic copolyamide; chitosan; chitin nanofibrils; hyaluronic acid hydrogel.

ВОЗМОЖНОСТЬ ПРИМЕНЕНИЯ НИЗКОТЕМПЕРАТУРНОЙ АТМОСФЕРНОЙ ПЛАЗМЫ И БИОПОЛИМЕРНЫХ РАНЕВЫХ ПОКРЫТИЙ ДЛЯ ЛЕЧЕНИЯ ОЖОГОВ КОЖИ III СТЕПЕНИ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

© Е.В. Зиновьев[1], М.С. Асадулаев[1], И.А. Комиссаров[1], М.В. Шемет[2], В.Е. Юдин[2], Р.Г. Стояновский[1], Н.В. Смирнова[2], А.С. Шабунин[2], С.А. Лукьянов[1], Т.А. Шалоня[1], А.Е. Крюков[2], И.В. Арцимович[1], Д.В. Костяков[1]

[1] ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России;
[2] ФГАОУ ВО «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург

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В статье приведены результаты применения низкотемпературной холодной атмосферной плазмы, а также разрабатываемых перспективных раневых покрытий на основе нановолокон хитозана и сополиамида, коммерческого гистеобиопластического материала на основе гидрогеля гиалуроновой кислоты при лечении ожогов кожи III степени (МКБ-10) в эксперименте. В ходе первого этапа исследования была разработана оригинальная методика для воспроизведения термического ожога кожи у мелких лабораторных животных (грызунов). Установлено, что применение низкотемпературной холодной атмосферной плазмы позволяет снизить частоту развития гнойных осложнений, а также способствует сокращению сроков восстановления кожного покрова на 20 % ($p < 0,05$), но не обеспечивает достижения результата, констатируемого после выполнения ранних некрэктомий и замещения дефектов гистеопластическими материалами на основе природных полимеров. Показано, что раневые покрытия на основе алифатического сополиамида и хитозана, а также гидрогеля гиалуроновой кислоты позволяют достоверно ускорить процессы репаративной регенерации и гистеогенеза в зоне термического ожога кожи после некрэктомии на 14,6–46 % (p < 0,05).

Ключевые слова: глубокие термические ожоги кожи; низкотемпературная атмосферная плазма; раневые покрытия; восстановление кожного покрова; алифатический сополиамид; хитозан; нанофибриллы хитина; гидрогель гиалуроновой кислоты.

INTRODUCTION

Modern tactics of treating burns are multidisciplinary, and are implemented taking into account the pathogenesis of burn disease and its complications [1, 2]. Modern methods of treating deep thermal skin damages do not allow for curing victims with critical lesions in all cases, leaving several unresolved issues, primarily in terms of choosing a quick and effective method of restoring the skin in such lesions [3, 4, 9]. To date, at burn hospitals, active surgical tactics are used in which the basic framework is early necrectomy followed by burn wound autothermografting [2, 10]. Prevention of infection with burns is especially important [5, 12]. One possible way to improve treatment results and increase the effectiveness of methods for restoring the skin in patients with extensive deep burns is the use of natural polymers. These nanobiocomposite polymers are combined with methods of physical impact on the wound surface, in particular, the use of low-temperature atmospheric plasma [6, 7, 11, 13, 15].

The biological effects of low-temperature low-pressure atmospheric plasma include antimicrobial and hemostatic effects, as well as stimulation of tissue regeneration [8, 14]. The antibacterial action is due to the damage to the cell wall and membrane of bacteria by ultraviolet spectrum radiation and active radicals [14]. In contrast to the physical impact of existing methods to arrest bleeding (electrocoagulation, argon-plasma coagulation), low-temperature atmospheric plasma does not damage tissues, but provides hemostasis due to accelerated activation and aggregation of platelets and formation of a fibrin clot. The issue of the direct influence of a low-temperature low-pressure atmospheric plasma on tissue regeneration in cases of damage remains disputable [14, 16]. Some researchers have noted the acceleration of fibroblast proliferation *in vitro* under such exposure. Some reports have explained the stimulation of regeneration of damaged tissues by the combination of antibacterial and hemostatic effects of plasma [14, 16].

Thus, the current data suggest that the use of low-temperature low-pressure atmospheric plasma can be promising for the treatment of deep skin burns. We investigated the effect of low-temperature atmospheric plasma and experimental wound coatings on reparative histogenesis in deep skin burns.

MATERIALS AND METHODS

We studied 40 male Wistar-Kyoto rats weighing 230 to 250 g. All manipulations were performed with the animals under general inhalation (ether) anesthesia under aseptic conditions. Reproduction of a third degree skin burn (ICD-10) was performed according to our own original method (the rationalization proposal of the Military Medical Academy No. 14287/1 of 01/19/2016). After preparation of the surgical field, the animal was fixed to the laboratory table. The area of the burn was marked with a stencil of 16 cm^2 (10% of the body area of the rat). On the depilated skin of the animal's back, the temperature of the skin and metal plate heated through the resistive heating element was determined using the electrothermocouple sensor of the Electroline multimeter (China; Figs. 1, 2). The exposure time was 10 seconds at a skin surface temperature of 95 °C to 97 °C (Figs. 3, 4).

To generate low-temperature low-pressure atmospheric plasma, we used a device made by the specialists of the Department of High Voltage, Electrical Insulation, and Cable Technology of the Institute of Energy and Transport Systems of Peter the Great St. Petersburg Polytechnic University (SPbPU). The device can be hand-held, and the contact of the plasma beam and the biological object does not lead to an electrophysical effect of the current. The device is powered by a constant current source. The output voltage can be altered to within 20 kV. The current source is connected in se-

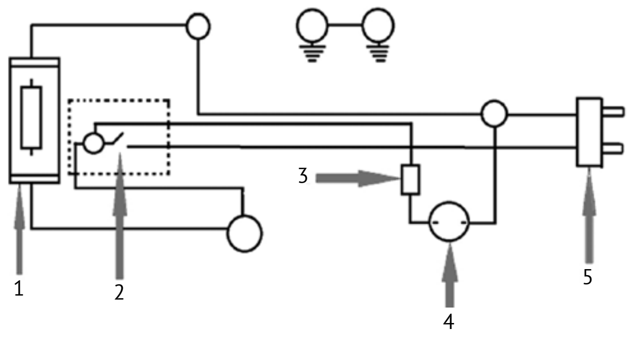

Fig. 1. Circuit of the heating element for reproducing the thermal burn. 1, the heating element; 2, the thermostat; 3, the resistor; 4, the indicator lamp; 5, the main plug

Рис. 1. Схема нагревательного элемента для воспроизведения термического ожога. 1 — нагревательный элемент, 2 — терморегулятор, 3 — резистор, 4 — лампа индикатора, 5 — сетевая вилка

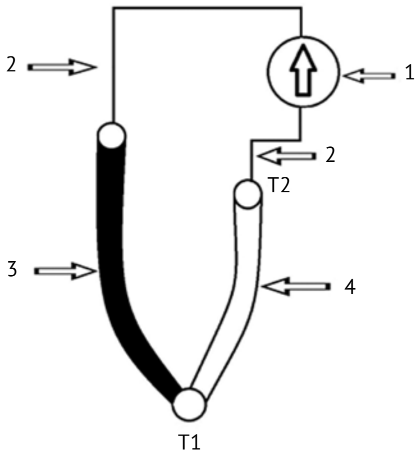

Fig. 2. Thermocouple circuit for high-temperature exposure dosage. 1, measuring device; 2, connecting wires; 3, 4, thermoelectrodes

Рис. 2. Схема термопары для дозировки высокотемпературного воздействия. 1 — измерительный прибор, 2 — соединительные провода, 3, 4 — термоэлектроды

Fig. 4. Skin of the rat after the reproduction of the third degree burn

Рис. 4. Кожа крысы после воспроизведения ожога III степени

ries with a steel needle electrode (tip diameter, 50 μm) through a 120 MΩ resistor (Fig. 3). When the generator is started, 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.

The animals were divided into five groups (eight individuals each) based on the method of treatment. In group 1, necrectomy was performed 60 min after the third degree burn (Fig. 5) to its fascia. Immediately after necrectomy, the wound edges were fixed to the subjacent tissues with interrupted sutures. Then, the entire wound surface was treated with low-temperature atmospheric plasma for 10 min. The distance between the beam source and wound surface was 0.5 to 1 cm (Fig. 6).

In groups 2 and 3, 60 min after the trauma, radical necrectomy to the fascia and application of wound cov-

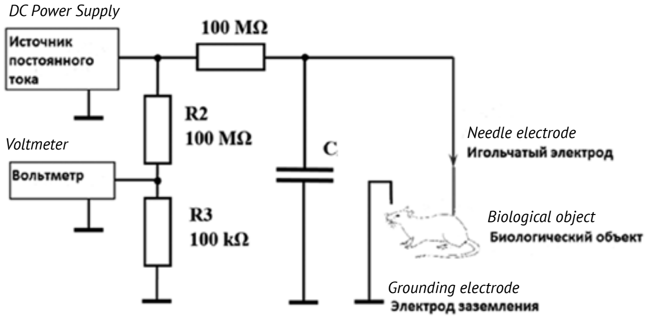

Fig. 3. Generator circuit of low-temperature low-pressure atmospheric plasma

Рис. 3. Схема генератора низкотемпературной атмосферной плазмы низкого давления

Fig. 5. Wound surface after necrectomy

Рис. 5. Раневая поверхность после выполнения некрэктомии

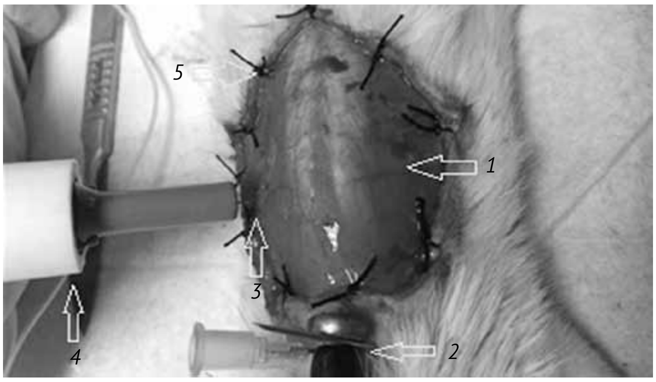

Рис. 6. Stage of the wound surface treatment with low-temperature atmospheric plasma: *1*, wound surface; *2*, grounding; *3*, cold atmospheric low-temperature plasma beam; *4*, handle of the device with insulation; *5*, single musculocutaneous sutures to prevent contraction of wounds

Рис. 6. Этап обработки раневой поверхности низкотемпературной атмосферной плазмой: *1* — раневая поверхность; *2* — заземление; *3* — пучок холодной атмосферной низкотемпературной плазмы; *4* — ручка аппарата с изоляцией; *5* — одиночные кожно-мышечные швы для предупреждения контракции ран

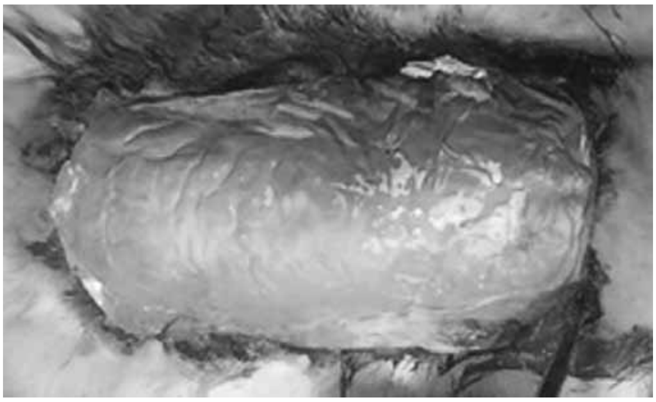

Fig. 7. Application of a wound coating based on aliphatic copolyamide and chitosan
Рис. 7. Аппликация раневого покрытия на основе алифатического сополиамида и хитозана

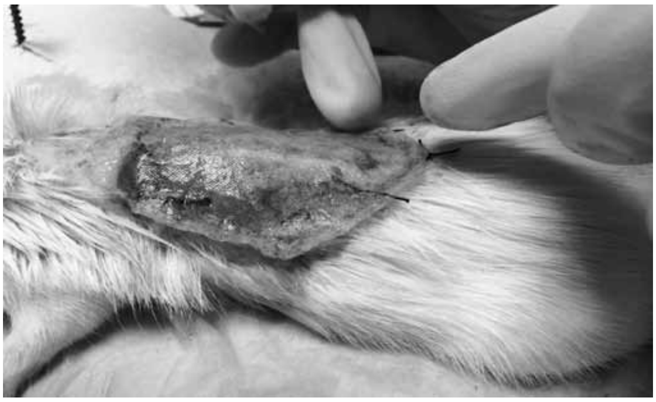

Fig. 8. Fixation of a wound coating based on hyaluronic acid hydrogel
Рис. 8. Фиксация раневого покрытия на основе гидрогеля гиалуроновой кислоты

erings on the basis of natural polymers were performed (Figs. 5–8). In group 2, the wounds were treated with the use of experimental wound coatings based on aliphatic copolyamide and chitosan produced by SPbPU (Fig. 7). In group 3, the wound surface was replaced with wound coatings based on the hydrogel of hyaluronic acid produced by LLC G-group, RF (Fig. 8). They were fixed additionally with Dermabond skin glue (Germany).

In group 4, early necrectomy and wound treatment were not performed (control). In group 5, early necrectomy was performed without wound treatment (control 2).

The efficacy of the selected treatment methods was evaluated and wounds were photographed every three days. The wounds were inspected and the nature of the discharge, and presence and type of granulation were noted. The timing of rejection of the scab and healing of wound surfaces was recorded. The wound area was determined with the planimetric method of Popova, and the healing index was calculated using the following formula (Fenchin K.I., 1979):

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

where S is the area of the wound at the previous measurement in mm^2, S is the area of the wound for the given measurement in mm^2, and T is the interval between measurements in days.

Biopsy specimens for histological examination were selected on days 3, 7, 12, 15, 21, and 28 of treatment. The biopsy specimens were fixed in 10% neutral formalin, followed by passage through ascending concentrations of alcohol (30% to 100%) and poured into paraffin. Paraffin sections were stained with hematoxylin and eosin, and investigated further with light-optical microscopy.

Processing of the obtained results was conducted in accordance with the conventional methods of variational statistics. $P < 0.05$ was considered indicative of statistical significance.

RESULTS

Radical surgical necrectomy in the deep skin burn zone without subsequent treatment led to a reduction of the wound area to 8 cm^2 on day 21 ($P < 0.05$). The regeneration process was accelerated by 8.6%, and by day 28 the scar area was decreased by 10% ($P < 0.05$) compared to the control group.

The process of regeneration in the deep zone of third degree burns treated with low-temperature atmospheric low-pressure plasma had significant special results. On day 21 of the study, the wound area was reduced to 6 cm^2 in these animals ($P < 0.05$). Treatment of a burn wound after early necrectomy resulted in acceleration of the regeneration processes in the early postoperative period by 20% ($P < 0.05$), and a reduction of the scar area by 52.5% on day 28 of observation ($P < 0.05$) compared to the control group (Fig. 9, Table 1).

Early necrectomy and the subsequent use of wound coatings based on aliphatic copolyamide and hyaluronic acid were more effective. When the defect was replaced with chitosan and copolyamide coating at the end of week 3 of the study, the wound area was reduced to 2.8 cm^2 ($P < 0.05$) in these animals. On day 28 of observation, the regeneration processes was accelerated by 42.8% ($P < 0.05$) and the scar area was reduced by 65% ($P < 0.05$) compared to the control group. When using hyaluronic acid-based coatings for wound closing after necrectomy, on day 28 of observation, the wound area was reduced to 5 cm^2 ($P < 0.05$) compared to the control group, the regeneration processes was accelerated by 14.3% ($P < 0.05$), and the scar area was reduced by 71.3% ($P < 0.05$). By this time, the largest defect

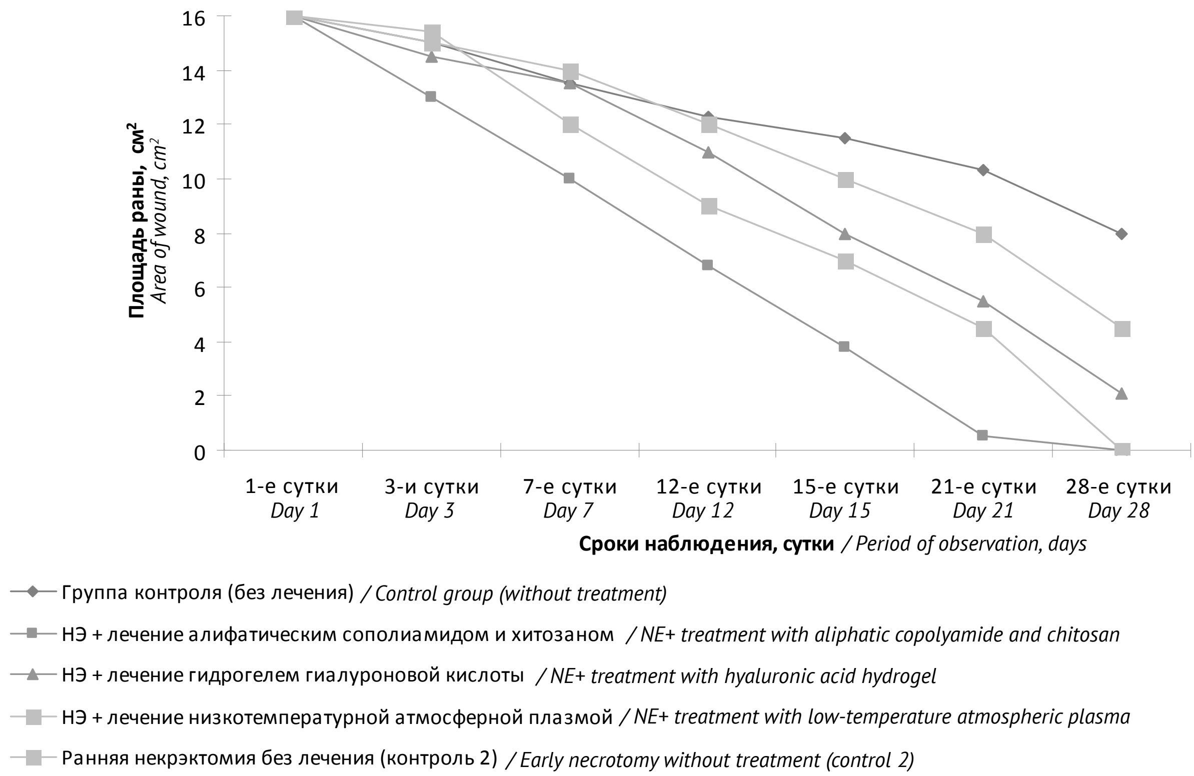

Fig. 9. Dynamics of the burn wound area, taking into account the method of treatment
Рис. 9. Динамика площади ожоговой раны с учетом метода лечения

Table 1

Planimetric evaluation of wounds, taking into account the methods of treatment

Таблица 1

Планиметрическая оценка ран с учетом методик лечения

Study groups	Healing period, days	Scar area, cm²
Control group	35 ± 2.9	5 ± 0.1
Low-temperature atmospheric plasma + NE	28 ± 2.1	3.8 ± 0.4*
Chitosan-copolyamide + NE	20 ± 3.4*	2.8 ± 0.7*
Hyaluronic acid + NE	30 ± 0.8**	2.1 ± 0.4*
NE without treatment (control 2)	32 ± 1.6**	4.5 ± 0.8

Примечание: НЭ — некрэктомия; * достоверно ($p < 0.05$) по сравнению с животными контрольной группы; ** достоверно ($p < 0.05$) по сравнению с хитозан-сополиамидом после некрэктомии
Note: NE is necrectomy; * significant ($P < 0.05$) compared to the animals in the control group; ** significant

area (8 cm²) was noted consistently in the control group, in which no treatment was performed.

The results of planimetric studies were confirmed by a morphometric evaluation of the number of vessels in the microcirculatory bed in the biopsy specimens by the end of day 35 of observation. The analyzing index was 46.2% greater in the animals that underwent surgical necrectomy and subsequent treatment of wounds with wound coatings based on hyaluronic acid hydrogel ($P < 0.05$). An average of five microvessels occurred in growing granulation tissues during wound treatment with low-temperature atmospheric plasma (Fig. 10). Early necrectomy without subsequent treatment resulted in an increase in the number of vessels by 13.4% compared to the control group ($P < 0.05$).

Morphometric evaluation on day 35 of observation showed that the thickness of the newly formed granulation tissue in the defect area was 1145 μm when the wound surface was treated with low-temperature atmospheric plasma, which was 4.8% higher than that in

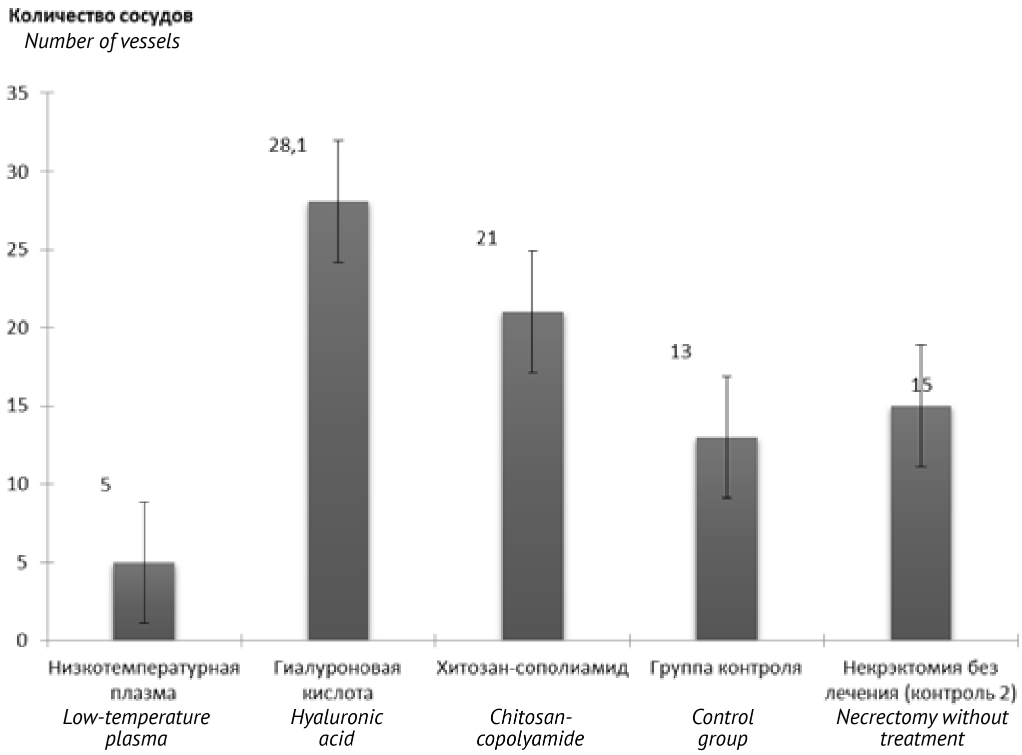

Fig. 10. The number of microvessels in the field of vision considering the method of treatment
Рис. 10. Число микрососудов в поле зрения с учетом метода лечения

Table 2

Thickness of newly formed granulations considering the method of treatment

Таблица 2

Толщина новообразованных грануляций с учетом метода лечения

Study groups	Tissue thickness, μm
Control group (without treatment)	1090.4 ± 25.9 ***
Treatment with aliphatic copolyamide and chitosan	1676.2 ± 67.1 *, ***
Treatment with hyaluronic acid hydroalgel	1273.4 ± 49.7 *
Treatment with low-temperature atmospheric plasma	1145.1 ± 44.3 **
Early necrectomy (without treatment)	1100.4 ± 71.7

Примечание: * достоверно (p < 0,05) по сравнению с контролем; ** достоверно (p < 0,05) по сравнению с хитозаном/сополиамидом после некрэктомии; *** достоверно (p < 0,05) по сравнению с гиалуроновой кислотой после некрэктомии
Note: * Significant (P < 0.05) compared to the control group; ** Significant (P < 0.05) compared to chitosan/copolyamide after necrectomy; *** Significant (P < 0.05) compared to hyaluronic acid after necrectomy

the control group (P < 0.01). Compared to the control group, the thickness increased by 1% in animals that underwent early necrectomy without subsequent treatment, and by 17% and 37% in animals that underwent early closure of the wound surface after necrectomy and subsequent wound management using chitosan/copolyamide and hyaluronic acid coatings, respectively. We concluded that the use of wound coverings ensures earlier development of a full-fledged connective tissue in deep thermal burn zone (Table 2).

CONCLUSIONS

Our results indicate that the use of low-temperature atmospheric plasma without further application of wound coverings enabled wound healing by day 28, which corresponds to an acceleration of the regeneration processes by 20% (P < 0.05) and a reduction of the scar area by 52.5% (P < 0.05) compared to the control group. When using low-temperature atmospheric plasma, earlier development of mature connective tissue was noted in biopsy specimens, and the thickness of newly formed granulation tissues exceeded that of controls by 4.8% ($P < 0.01$). Early necrectomy without subsequent treatment resulted in a reduction of the wound area on day 21 to 8 cm^2, the regeneration process was accelerated by 8.6% ($P < 0.05$), and by day 28 the scar area was decreased by 10% ($P < 0.05$) compared to the control group. The use of wound coatings based on aliphatic copolyamide and chitosan in the deep thermal burn zone after necrectomy resulted in complete wound healing already by day 20, accelerating the regeneration processes by 42.8% ($P < 0.05$), and decreasing the scar area by 65% ($P < 0.05$). The use of wound coatings based on hyaluronic acid hydrogel under the same conditions resulted in a shortened healing time (14.3%, $P > 0.05$) and a decreased scar area (71.3%, $P < 0.05$).

The use of low-temperature atmospheric plasma for treatment of third degree deep thermal skin burns (ICD-10) is a promising method. Data from the literature indicate that the topical application of low-temperature low-pressure atmospheric plasma in the skin defect zone allows for implementation of antimicrobial and hemostatic action, and stimulation of tissue regeneration. These phenomena are due to the generation of free radicals, UV radiation, and charged particles [8, 14, 16]. Our results showed that the use of plasma in the deep zone of third degree burns resulted in reduction of the scar area by 52.5% ($P < 0.05$), but did not parallel the result obtained after early necrectomy. Early necrectomy and one-stage wound surface closure with a wound coating based on natural polymers reliably shortened healing time. In the next series of experiments, we plan to evaluate the efficacy of the combination of topical application of plasma after necrectomy with the use of wound coverings.

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◆ Information about the authors

Evgeny V. Zinovyev — MD, PhD, Dr Med Sci Professor, Department of Hospital Surgery with Traumatology and Military Surgery Courses. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: evz@list.ru.

Marat S. Asadulaev — Department of Surgical Diseases of Childhood. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: marat.asadulaev@yandex.ru.

Igor A. Komissarov — MD, PhD, Dr Med Sci Professor, Head, Department of Surgical Diseases of Childhood. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: komissarov_i_a@mail.ru.

Mihail V. Shemet — PhD, Associate Professor. Department of High Voltage Engineering, Electrical Insulation and Cable Technology, Peter the Great St Petersburg Polytechnic University, Saint Petersburg, Russia. E-mail: mvshemet@gmail.com.

Vladimir E. Yudin — Dr Sci Professor, Head. Institute of Physics, Nanotechnology and Telecommunications, Department of Medical Physics, Laboratory of Polymeric Materials for Tissue Engineering and Transplantology, Peter the Great St Petersburg Polytechnic University, Saint Petersburg, Russia. E-mail: yudinve@gmail.com.

Anton S. Shabunin — Institute of Physics, Nanotechnology and Telecommunications, Department of Medical Physics, Laboratory of Polymeric Materials for Tissue Engineering and Transplantology, Peter the Great St Petersburg Polytechnic University, Saint Petersburg, Russia. E-mail: anton-shab@yandex.ru.

◆ Информация об авторах

Евгений Владимирович Зиновьев — д-р мед. наук, профессор, кафедра госпитальной хирургии с курсами травматологии и ВПХ. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: evz@list.ru.

Марат Сергеевич Асадулаев — кафедра хирургических болезней детского возраста. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: marat.asadulaev@yandex.ru.

Игорь Алексеевич Комиссаров — д-р мед. наук, профессор, заведующий, кафедра хирургических болезней детского возраста. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: komissarov_i_a@mail.ru.

Михаил Вячеславович Шемет — канд. техн. наук, доцент. Институт энергетики и транспортных систем, кафедра техники высоких напряжений, электроизоляционной и кабельной техники, ФГАОУ «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург. E-mail: mvshemet@gmail.com.

Владимир Евгеньевич Юдин — д-р физ.-мат. наук, профессор, заведующий. Институт физики, нанотехнологий и телекоммуникаций, кафедра медицинской физики, лаборатория полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург. E-mail: yudinve@gmail.com.

Антон Сергеевич Шабунин — Институт физики, нанотехнологий и телекоммуникаций, кафедра медицинской физики, лаборатория полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург. E-mail: anton-shab@yandex.ru.

◆ Information about the authors

Natalia V. Smirnova — PhD Researcher. Institute of Physics, Nanotechnology and Telecommunications, Department of Medical Physics, Laboratory of Polymeric Materials for Tissue Engineering and Transplantology, Peter the Great St Petersburg Polytechnic University, Saint Petersburg, Russia. E-mail: nvsmirnoff@yandex.ru.

Artem E. Kryukov — Institute of Physics, Nanotechnology and Telecommunications, Department of Medical Physics, Laboratory of Polymeric Materials for Tissue Engineering and Transplantology, Peter the Great St Petersburg Polytechnic University, Saint Petersburg, Russia. E-mail: kryukov14@gmail.com.

Sergey A. Lukyanov — Department of Surgical Diseases of Childhood. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: stalker125lu@yandex.ru.

Roman G. Stoyanovskiy — Assistant Professor, Department of Human Anatomy. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: explabgpmu@gmail.com.

Tatiana A. Shalonia — MD, PhD, Assistant Professor, Department of Pathological Anatomy at the Rate of Forensic Medicine. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: tanyaiudina@mail.ru.

Ilya V. Artsimovich — Department of Surgical Diseases of Childhood. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: enerdgezer@yandex.ru.

Denis V. Kostyakov — Department of Pathologic Physiology Courses Immunopathology and Medical Informatics. St Petersburg State Pediatric Medical University, Ministry of Healthcare of the Russian Federation, Saint Petersburg, Russia. E-mail: kostyakovdv@gmail.com.

◆ Информация об авторах

Наталья Владимировна Смирнова — канд. биол. наук, научный сотрудник. Институт физики, нанотехнологий и телекоммуникаций, кафедра медицинской физики, лаборатория полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург. E-mail: nvsmirnoff@yandex.ru.

Артем Евгеньевич Крюков — Институт физики, нанотехнологий и телекоммуникаций, кафедра медицинской физики, лаборатория полимерных материалов для тканевой инженерии и трансплантологии, ФГАОУ «Санкт-Петербургский политехнический университет Петра Великого», Санкт-Петербург. E-mail: kryukov14@gmail.com.

Сергей Андреевич Лукьянов — кафедра хирургических болезней детского возраста. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: stalker125lu@yandex.ru.

Роман Григорьевич Стояновский — ассистент, кафедра анатомии человека. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: explabgpmu@gmail.com.

Татьяна Александровна Шалоня — канд. мед. наук, ассистент, кафедра патологической анатомии с курсом судебной медицины. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: tanyaiudina@mail.ru.

Илья Валерьевич Арцимович — кафедра хирургических болезней детского возраста. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: enerdgezer@yandex.ru.

Денис Валерьевич Костяков — кафедра патологической физиологии с курсами иммунопатологии и медицинской информатики. ФГБОУ ВО «Санкт-Петербургский государственный педиатрический медицинский университет» Минздрава России, Санкт-Петербург. E-mail: kostyakovdv@gmail.com.