

DOI: 10.34921/amj.2023.4.008

AŞAĞI ƏTRAFLARDA OLAN XRONİK YARALARIN VAKUUM-TERAPİYA ÜSULU İLƏ MÜALİCƏSİ: KLİNİK ƏLAMƏTLƏRİN VƏ BİOLOJİ ZARLARIN GÖSTƏRİCİLƏRİNİN TƏHLİLİ

I.V.Korpusenko¹, N.N.Nor¹, V.P.Krişen¹, N.V.Trofimov¹, Y.G.Garkavaya⁴, Y.İ.Korpusenko¹,
A.N.Makarenko², V.E.Kudryavtseva³, V.V.Koştura¹

¹Dnepr Dövlət Tibb Universiteti, Dnepr, Ukrayna;

²Regionlararası Şəxsi Heyətin İdarə edilməsi Akademiyası, Kiyev, Ukrayna;

³Dnepryanı Dövlət Bədən Tərbiyəsi və İdman Akademiyası, Dnepr, Ukrayna;

⁴Milli Aviasiya Universiteti, Kiyev, Ukrayna

Xülasə. Məqalədə xronik yaralarda olan bioloji zarlara mənfi atmosfer təzyiqinin təsirini öyrənmək məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir. Tədqiqata aşağı ətraflarında olan xronik yaraya görə müayinə və müalicə almış, yaşı 45-lə 75 arasında olan xəstə cəlb edilmişdir. Mikroorqanizmlərin identifikasiyası üçün MALDI-TOF spektroskopiyaya ("Bruker" Almaniya firmasının istehsalı olan aparat vasitəsilə) üsulundan istifadə edilmişdir. Bioloji zarların əmələ gəlməsinin rəqəmli parametrləri Stat Fax 2100 fotometri vasitəsilə müəyyənləşdirilmişdir.

Xəstələrdən 72%-nin xronik yaralarında mikrob zarları müşahidə edilmişdir. Xronik infeksiyanın törədiciləri arasında Gram-mənfi və Gram-müsbət bakteriyaların müxtəlif növləri aşkar edilmişdir, ən çox yayılmış mikroorqanizmlər isə Stafilokoklar qrupuna aid olmuşdur. Vakuüm-terapiyanın cərrahi müalicə və antibakterial terapiya ilə birgə tətbiqi bioloji zarların bərpasının qarşısını almış və yaraların sonrakı infeksiyalaşmasını aradan qaldırmışdır. Bu isə xronik yara probleminin radikal həllinə imkan verir və müalicə alan xəstələrdə yaraların sağlamlasına 100 % -li şərait yaradır.

Açar sözlər: bioloji zarlar, xronik xora, vakuüm-terapiya, cərrahi müalicə

Ключевые слова: биопленки, хроническая рана, VAC-терапия, хирургическое лечение

Key words: biofilms, chronic wound, VAC therapy, surgical treatment

VAC-THERAPY IN THE TREATMENT OF LOWER LIMB CHRONIC WOUNDS. ANALYSIS OF CLINICAL AND BIOFILM DATA

I.V.Korpusenko¹, N.N.Nor¹, V.P.Kryshen¹, N.V.Trofimov¹, E.G.Garkava⁴,
E.I.Korpusenko¹, A.N.Makarenko², V.E.Kudryavtseva³, V.V.Koshtura¹

¹Dnipro State Medical University. Dnieper. Ukraine

²Interregional Academy of Personnel Management. Kyiv. Ukraine.

³Pridneprovsk State Academy of Physical Culture and sports Dnieper. Ukraine

⁴National Aviation University. Kyiv, Ukraine

The article presents the results of a study conducted to investigate the effects of negative atmospheric pressure on the biofilms of chronic wounds. A total of 62 patients with chronic wounds of the lower extremities, aged 45 to 75 years, were examined and treated. Microorganism identification was performed using MALDI-TOF spectrometry (Bruker, Germany). The quantitative expression of biofilm formation was represented by optical density values measured on the StatFax2100 photometer. Microbial biofilms were detected in 72% of patients with chronic wounds. The causative agents of chronic infection included representatives of both Gram-positive and Gram-negative flora, with a tendency for Staphylococcus species to predominate. VAC therapy, in combination with surgical treatment and antibiotic therapy, prevented the reformation of biofilm, hindered further wound infection, leading to a radical resolution of the wound process, and resulted in wound healing in 100% of treated patients.

Biofilms are ubiquitous in nature because it is part of bacteria's own survival mechanism [1]. As early as 1683, Antoni van Leeuwenhoek observed and described biofilms using his primitive microscope on material from his teeth. However, the biofilm lifestyle of microorganisms was not of interest to medical microbiologists until the early 1970s, when Nils Høiby discovered a connection between the etiology of persistent infection and bacterial aggregates in patients with cystic fibrosis [2]. Since then, it has been recognized that biofilms are involved in many chronic infections [2,3] and, when accumulated, influence their pathogenesis [4,5,6].

Biofilms are complex colonies of bacterial populations (bacteria and fungi) enclosed in a protective extracellular polymeric substance (matrix) that helps them adhere to a suitable surface [6]. The main components of the biofilm matrix are water (up to 97%), microbial cells (up to 5%), polysaccharides, proteins and nucleic acids (up to 2% of each component). [7]. Bacteria in biofilms acquire a special form of antibiotic resistance, which is manifested in increased resistance to antimicrobial agents, disinfectants and immune defense of the macroorganism.

Research over the past two decades has established the pervasive role of biofilm in the formation of chronic wounds [8]. An analysis of published data indicates a prevalence of biofilms of 78.2% in chronic wounds, which poses a significant threat to their healing [9]. One of the promising methods for treating wounds of various etiologies is vacuum therapy, negative pressure wound therapy – NPWT, vacuum assisted closure – VAC), which is one of the types of local treatment that is used to optimize the course of the wound process [10,11]. In recent years, significant progress has been made in the study and use of VAC-therapy, however, the mechanisms of influence of this method on various parts of the wound process require further research.

The purpose of the study: improve the results of treatment of patients with chronic wounds of the lower extremities by using complex treatment, including surgical interventions, VAC- therapy, and also studying the effect of negative pressure on wound biofilms.

Materials and methods. During the period from 2018 to 2023 in the clinic of the Department of General Surgery, Surgery No. 3, Traumatology and Orthopedics of the Faculty of Professional Education of the Dnieper Medical University, 62 patients with chronic wounds of the lower extremities were examined and treated. These are mostly women (58) aged 45 to 75 years. The area of the wounds ranged from 5 to 30 cm in diameter. Among the causes, 28 (45.2%) were complications of chronic venous insufficiency, in 18 (29%) the cause of wounds was mechanical injuries, in 8 (12.9%) – animal and insect bites; in 5 (8.1%) – consequences of acute purulent diseases and in 3 (4.8%) – bedsores. The effectiveness of treatment was assessed based on the study of clinical indicators of wound healing and microbiological examination. Microbiological studies were carried out upon admission of patients to the hospital and on the 14th day after VAC -therapy. In the operating room, after treating the wound with an antiseptic Betadine solution, an area of the wound surface was excised. The biopsy specimen was ground in a mortar with sterile saline solution at a ratio of 1:10. Tenfold dilutions were prepared and inoculated using the Gouldie method on blood nutrient agar plates, followed by quantitative recording of microbial contamination [13]. Identification of microorganisms was carried out using MALDI-TOF spectrometry (Bruker, Germany). Quantitative assessment of the ability of microorganisms to form biofilms was carried out using the photometric method in 96-well plates for enzyme immunoassay [14]. To study the ability to form biofilms, one-day cultures of *Staphylococcus aureus* and *Escherichia coli* isolated from patients were used. The quantitative expression of the degree of biofilm formation was the optical density values measured on a StatFax2100 photometer.

The S-300 VAC system was used to treat wounds with negative pressure. During the first installation of the system, the device was set to a constant aspiration mode with a negative pressure in the wound cavity of 80-110 mm Hg. Art. Vacuum dressings were used (hydrophilic polyurethane sponges with a pore size of 400–2000 µm with a transparent adhesive coating, connected by a drainage tube to a vacuum source apparatus). The first period of system operation lasted from 24 to 48 hours, the second up to 3 days, the subsequent ones - on average up to 5 days. The system was remounted for the purpose of wound revision and during surgical treatment in the operating room.

Statistical processing of the data was carried out by determining the arithmetic mean (M) and its error (m). The significance of the difference in mean values was determined using the Student's t test.

Statistical data processing was carried out using a personal computer using STATISTICA 6.1 software (StatSoftInc., serial AGAR909E415822FA) and Microsoft Excel (Microsoft Office 2016 Professional Plus, Open License 67528927) using descriptive and analytical biostatistics methods and multivariate statistical analysis methods.

Results. Complex treatment began with VAC-therapy, both to prepare wounds for surgery and to reduce tissue swelling, stimulate blood flow and cleanse necrotic tissue. VAC-therapy was also used after autodermoplasty surgery (Fig. 1).

After completing the course of negative pressure treatment in the postoperative period, dressings with ointments with hyaluronic acid were used: Cicatridina, Ialuset –for 2-3 weeks. Treatment was carried out under the condition of adequate unloading of the limbs. Targeted antibacterial therapy was carried out against the background of VAC -therapy and complex surgical treatment. The course of treatment

was carried out in accordance with the results of bacteriological cultures and the choice of drugs to which the greatest sensitivity was identified.

The use of VAC-therapy contributed to the rapid cleansing of wounds, reducing their area and depth, accelerated formation of granulations and epithelization of edges, and reduced costs for wound care products. The reduction in local phenomena of the inflammatory process occurred much faster and more effectively than when using standard local treatment (ointment dressings, wound coverings). This is confirmed by positive changes in the local status on days 4–5 of treatment: a decrease in hyperemia, tissue edema, as well as the amount of wound exudate, a change in its nature from purulent to serous. Signs of epithelization of the wound edges appeared, the tissue defect was gradually filled with granulations and decreased in size (Fig. 2).



Fig.1. Chronic wound of the left knee joint. VAC-therapy.



Fig.2. Chronic wound of the left leg: a) upon admission to the hospital; b) after VAC therapy.

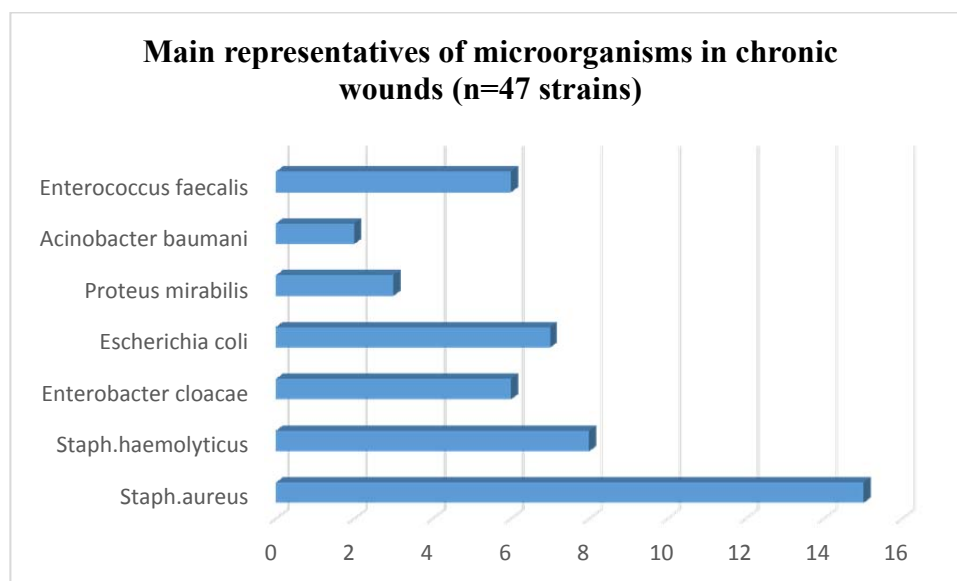


Fig.3. The main representatives of microorganisms in chronic wounds (n=47 strains)

The duration of treatment of patients in hospital was on average (38 ± 3) days. Complete wound healing was achieved in all patients. In 67% of patients, the transplanted autoderm graft took root completely. Partial lysis was observed in 28%, requiring reoperation. In 3% of cases, the wounds healed without surgery. In 2%, severe pain was observed during exposure to negative pressure, which forced the abandonment of VAC therapy and plastic surgery using a perforator propeller flap. Relapses were observed in 2 patients 1 year later and in 1 patient 2 years after treatment.

Microbiological monitoring was carried out upon admission and on the 14th day after VAC- therapy. 47 strains of pathogens were isolated (Fig. 3). The main representatives among the isolated microorganisms were Staphylococcus aureus (15 strains), coagulase-negative staphylococci Staphylococcus haemolyticus (8 strains). Enterobacter cloacae (6 strains) and Escherichia coli (7 strains) were found among enterobacteria; Enterococcus faecalis (6 strains), Proteus mirabilis (3 strains) and Acinetobacter baumannii (2 strains).

The contamination of the wound in the examined patients at the time of the initial examination averaged $2.6 \pm 0.03 \times 10^4$ CFU/ml. To examine the ability of isolated

microorganisms to participate in the formation of biofilm conglomerates consisting of bacteria and extracellular matrix, a photometric method was used. The intensity of biofilm formation was assessed 24 hours after introducing microbes into plastic plates with Mueller-Hinton broth. Biofilm formation was studied before the start of VAC- therapy and on the 14th day after exposure to negative pressure. Biofilms were found in 72% of patients with chronic wounds. A slight predominance of the activity of staphylococcal flora in the formation of biofilms was noted – 1.4-1.6 cu. e. wholesale pl. compared to E. coli - 1.2-1.4 cu. e. wholesale pl. before the start of treatment. On the 14th day after the start of VAC -therapy, a tendency towards a decrease in the intensity of biofilm formation was found: in S. aureus – 1.2-1.5 c.u. and for E. coli – 0.9-1.1 c.u. respectively. The VAC-therapy we used prevented the restoration of the biofilm and prevented further infection of the wound.

Discussion. Our knowledge of biofilm and its impact on wound healing, especially non-healing wounds, is still evolving. Clinically, a chronic wound with red, loose granulation tissue covered with a mucous layer of exudate that returns after debridement, with increased production of discharge, and signs of recession of the epithelial edge, most likely

has a biofilm [15]. In patients with chronic wounds, a long-lasting inflammatory phase, inflammatory mediators and proteases delay the onset of proliferation and disrupt the normal epithelialization process [16]. In the outer layers of the biofilm, bacteria are predominantly aerobic, actively metabolize, multiply rapidly and grow easily in cultures. Deep-lying bacteria can survive in low oxygen concentrations in an anaerobic environment, remain dormant for long periods of time, and do not grow well on conventional crops. A number of observations from laboratory and animal experiments have contributed to the understanding of the pathogenesis of delayed healing in biofilm-colonized chronic wounds. But because the biofilm itself is so complex, an individual bacterial model is not sufficient to simulate a truly complex clinical picture. The lack of human experiments due to ethical concerns provides little information about its effects on chronic wounds. The underlying pathophysiology of biofilm is thought to involve manipulating the host's inflammatory response and converting it primarily into a

constant source of nutrition. The inability of the host immune system to suppress hyperinflammation induces the conversion of the innate immune response into an adaptive immune response, which leads to arrest of wound healing in the inflammatory phase and inhibition of the proliferative phase and epithelialization. Thus, biofilm directly or indirectly affects all phases of wound healing and is currently considered as an independent factor in delaying the normal healing process.

Conclusions

1. Microbial biofilms were found in 72% of patients with chronic wounds. The causative agents of chronic infection were representatives of gram-positive and gram-negative flora with a tendency to predominance of representatives of the genus *Staphylococcus*.

2. VAC-therapy in combination with surgical treatment and proper antibacterial therapy prevented the restoration of biofilm, prevented further infection of wounds, which led to radical solution of the wound process and healing in 100% of treated patients.

ЛІТЕРАТУРА

1. Lene K. Vestby, Torstein Grønseth, Roger Simm, Live L. Nesse. Bacterial Biofilm and its Role in the Pathogenesis of Disease // *Antibiotics*, 2020. 9, 59; doi:10.3390/antibiotics9020059 www.mdpi.com/journal/antibiotics, 1-29.
2. Høiby N.A. short history of microbial biofilms and biofilm infections // *APMIS*, 2017. 125, 272–275.
3. Morton L.M., and Phillips T.J. Wound healing and treating wounds: differential diagnosis and evaluation of chronic wounds // *J. Am. Acad. Dermatol.* 2016. 74, 589–605. doi: 10.1016/j.jaad.2015.08.068 .
4. Krzyszczyk P., Schloss R., Palmer A., and Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. *Front // Physiol.* 2018. 9:419. doi: 10.3389/fphys.2018.00419
5. Bjarnsholt T. The role of bacterial biofilms in chronic infections // *APMIS*, 2013. 121, 1–58.
6. Roche ED, Renick PJ, Tetens SP et al. Increasing the presence of biofilm and healing delay in a porcine model of MRSA-infected wounds. 2012. *Wound Repair Regen*; 20:537–43.
7. Вринчану Н.О., Дудікова Д.М., Гринчук Н.І, Недашківська В.В.(2019) Біоплівки. Сучасний стан та перспективи антимікробної терапії // *Фармакологія та лікарська токсикологія*, Том 13, 5, 311-321. doi.org/10.33250.13.05.311. [Vrynchanu N.O., Dudikova D.M., Hrynchuk N.I., Nedashkivs'ka V.V. (2019) Bioplivky. Suchasnyy stan ta perspektyvy antymikrobnoyi terapiyi. *Pharmacology and Drug Toxicology*, 13 (5), 311–321 doi.org/10.33250.13.05.311]. [Ukraine]
8. Wolcott RD, Rhoads DD, Bennett ME et al.(2010) Chronic wounds and the medical biofilm paradigm // *J Wound Care* ;19:45–46; 48–50, 52–53.
9. Malone M, Bjarnsholt T, McBain AJ, et al. (2017) The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data // *J Wound Care*;26:20–25.
10. Черкасов М.Ф., Галашокян К.М., Старцев Ю.М., Черкасов Д.М., и соавт. Опыт лечения ран различной этиологии с применением вакуум-терапии // *Sciences of Europe* . 2019. 40, 6-11. [Cherkasov M.F., Galashokjan K.M., Starcev Ju.M., Cherkasov D.M., i soavt. Opyt lechenija ran razlichnoj jetiologii s primeneniem vakuuum-terapii // *Sciences of Europe* . 2019. 40, 6-11.]
11. Нор Н.М., Слесаренко С.В., Трофімов М.В., Слесаренко К.С., Корпусенко О.І.(2017). Застосування вакуум-терапії у комплексі лікування хронічних ран нижніх кінцівок. *Klinichna khirurgiia*; 3, 39-41. <https://repo.dma.dp.ua/2097/1/Untitled.FR11.pdf>. [Nor N.M., Slesarenko S.V., Trofimov M.V., Slesarenko K.S.,

- Korpusenko O.I.(2017). Zastosuvannja vakuum-terapii u kompleksi likuvannja hronichnih ran nizhnih kincivok. Klinichna khirurgiia; 3, 39-41. <https://repo.dma.dp.ua/2097/1/Untitled.FR11.pdf>]
12. Korpusenko I.V., Mor N.N., Kryshen V.P., Trofimov M.N., Kudryavtseva V.E., Guzenko A.N. et al. Clinical and immunological aspects of the treatment of chronic wounds using VAC-therapy // Azerbaijan Medical Journal, 2023, №3. p. 51-57
 13. Бойцов А.Г., Иванов В.П., Ластовка О.Н., Порин А.А.(1999) Введение в клиническую микробиологию М.,153р.[Boytsov A.G, Ivanov V.P., Lastovka O.N., Porin A.A.(1999) Vvedeniye v klinicheskuyu mikrobiologiyu; M.,153p. [Russia]]
 14. Тец Г.В., Артеменко Н.К., Заславская Н.В, Тец В.В.(2013) Состояние бактериальных биопленок при длительном культивировании // *Иммунология и микробиология*; 155,4, 460-463. [Tets G.V., Artemenko N.K., Zaslavskaya N.V, Tets V.V.(2013) Sostoyaniye bakterial'nykh bioplenok pri dlitel'nom kul'tivirovani. *Immunologiya i mikrobiologiya*; 155,4, 460-463. [Russia]]
 15. Lenselink E, Andriessen A.(2011) A cohort study on the efficacy of a polyhexanide-containing biocellulose dressing in the treatment of biofilms in wounds. J Wound Care ;20:536–9.
 16. Zhao G, Usui ML, Underwood RA, et al.(2012) Time course study of delayed wound healing in a biofilm-challenged diabetic mouse model // Wound Repair Regen ;20:342–52.

ВАК-ТЕРАПИЯ В ЛЕЧЕНИИ ХРОНИЧЕСКИХ РАН НИЖНИХ КОНЕЧНОСТЕЙ: АНАЛИЗ ДАННЫХ КЛИНИКИ И БИОПЛЕНОК

**И.В.Корпусенко¹, Н.Н.Нор¹, В.П.Крышень¹, Н.В.Трофимов¹, Е.Г.Гаркавая⁴,
Е.И.Корпусенко¹, А.Н.Макаренко², В.Е.Кудрявцева³, В.В.Коштура¹**

¹*Днепровский государственный медицинский университет, Днепр, Украина;*

²*Межрегиональная академия управления персоналом, Киев, Украина;*

³*Приднепровская государственная академия физической культуры и спорта, Днепр, Украина;*

⁴*Национальный авиационный университет. Киев. Украина*

Резюме. В статье представлены результаты исследования, проведенного с целью изучения действия отрицательного атмосферного давления на биопленки хронических ран. Обследовано и пролечено 62 больных с хроническими ранами нижних конечностей в возрасте от 45 до 75 лет. Идентификация микроорганизмов проводилась с использованием MALDI-TOF спектрометрии («Bruker», Германия). Количественным выражением степени биопленкообразования служили значения оптической плотности, измеряемые на фотометре StatFax2100.

Микробные биопленки обнаружены у 72% больных с хроническими ранами. Возбудителями хронической инфекции были представители грамположительной и грамотрицательной флоры с тенденцией преобладания представителей рода *Staphylococcus*. VAC-терапия в сочетании с хирургическим лечением и антибактериальной терапией предотвращала восстановление биопленки, предупреждала дальнейшее инфицирование ран, что привело к радикальному решению проблемы раневого процесса и заживлению ран у 100% лечившихся пациентов.

Autor for correspondence:

Nadezdha N.Nor: Dnipro state Medical University, Kyev Ukrayne

E-mail: nadenor@i.ua