

EFFICACY OF DISPERSED FIBROUS CARBON SORBENT IN TREATMENT OF MALIGNANT FUNGATING WOUNDS

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Aim: To evaluate the influence of dispersed fibrous carbon sorbent (DFCS) on malignant fungating wounds (MFWs) in Guerin's carcinoma-bearing rats with an assessment of wound bacterial microflora. **Materials and Methods:** The study was performed on female Wistar rats inoculated subcutaneously with Guerin T8 carcinoma into the upper interscapular region. On day 20, the tumors were infected by injecting the suspension of *S. aureus* 8325-4. After 24 h, gauze dressings were applied daily to the wounds in the control group and DFCS in the experimental one. The state of the wounds was assessed by visual control with photo monitoring, odor control on a verbal rating scale and microbiological analysis of qualitative and quantitative characteristics of wound microbiota. **Results:** On the 3rd day after the start of dressings, the number of *S. aureus* of 8325-4 strain was significantly higher in the wounds of rats in control group than experimental one (5.14 ± 0.27 vs 3.43 ± 0.33 lg CFU/ml). The total number of *Staphylococci* in the DFCS-treated wounds did not differ statistically from the number of *S. aureus* 8325-4 strain whereas in control ones were higher by an order of magnitude. On the 10th day the total number of *Staphylococci* and *S. aureus* 8325-4 in particular, remained consistently high in the wounds of the control rats, while was almost five orders of magnitude lower and represented mainly by *S. aureus* 8325-4 in the experimental ones. The total number of aerobic and optionally anaerobic microorganisms was significantly lower as well. A greater variety of microorganisms in the gauze-covered wounds, as well as an their increased number were accompanied by enhancement of the wound odor from "noticeable" to "strong". There was an appearance of a "barely noticeable" odor in only one animal from the experimental group. **Conclusion:** The study has demonstrated the ability of DFCS to control substantially the bacterial microflora as well as malodor of MFWs *in vivo*. The results obtained can contribute to solving the problem of improving the quality of palliative care for patients with malignant and other chronic wounds.

Key Words: adsorptive dressing means, carbon dressings, malignant fungating wounds, chronic wounds, wound microbiota, wound odor.

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The treatment of patients with malignant fungating wounds (MFWs), caused by infiltration or metastasis of a malignant tumor in the skin [1], to date is not only a pressing medical but also a socio-economic and humanitarian problem. The frequency of MFWs in patients with advanced cancer as well as with metastases is estimated as 5–10% [2, 3].

Patients with MFWs suffer from a devastating burden of wound-related symptoms (pain, malodor, copious exudate, bleeding, increased microbial growth and necrosis) [1, 4], which inevitably lead to serious psychological and emotional consequences for patients and their families [1, 5]. MFW management is mainly palliative and its goal is to minimize suffering and optimize the life quality of patients *via* the adequate control of the severe symptoms associated with them [6, 7]. A key works in this area are mainly devoted to the practical care for patients with MFWs and are intended for healthcare professionals and specialized sick-nurses [4, 8]. The results of analyzing the clinical data on the use of dressings and other topical agents for MFW treatment, were presented in the

systematic reviews of the Cochrane database [9], along with a positive assessment of certain of them (honey, carbon and silver dressings), highlighted the significant lack of researches in this area and the need in new local means for monitoring (controlling) severe manifestations of MFWs.

One of the most frequently reported MFWs manifestations is wound malodor that limits the social coexistence of the patients contributing to their social isolation and deterioration of life quality [1, 10]. That is why MFW eradication is considered one of the most important tasks [11, 12]. In this regard, it was required to find the relationship between odor and bacterial strains identified in malignant wounds [13]. The state of MFWs is significantly affected by wound microbiota, its qualitative and quantitative characteristics [14]. Anaerobic colonization of the wound significantly enhances the odor, the amount of exudate and the intensity of the pain. The presence of more than four different types of bacteria in the microbiota of MFWs increases the risk of odor and exudation by more than 80%.

The odor that accompanies malignant wounds is associated with a number of compounds and dimethyltrisulfide primarily, as well as phenol, indole, short-chain fatty acids, volatile metabolites such as cadaverine, putrescine, sulfur, etc., produced in the process of the life of microorganisms inherent in MFWs [15]. The strongest and most typical for malign-

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Abbreviations used: ACD – adsorptive carbon dressing; ADM – adsorptive dressing means; DFCS – dispersed fibrous carbon sorbent; MFWs – malignant fungating wounds.

nant wound odor was found to be produced by *Proteus mirabilis*, *Staphylococcus aureus* and *Fusobacterium necrophoru*. Favorable microenvironments for the reproduction of pathogenic microorganisms are created by insufficient vascularization, necrosis and destruction of tissues, accumulation of exudate and tissue detritus in the wound [10].

Thus, the close association between the bacterial burden in wound and the intensity of odor, as well as the severity of other symptoms of MFWs, dictates the need to develop new antimicrobial strategies, one of which is the creation of local agents for the effective adsorption of volatile compounds, and to eliminate the main cause of their occurrence — bacteria causing the odor.

Charcoal based dressings remain one of the relevant local means used today for this purpose, although an international survey of 1,444 doctors in more than 30 countries found that only 48% of them reported dressing's effectiveness [16].

Adsorptive dressing means (ADM) on the base of activated carbon fibrous materials, including an adsorptive carbon dressing (ACD) and a dispersed fibrous carbon sorbent (DFCS), have been developed in R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine and are being used for many years to treat wounds and burns. Their effectiveness has been proven in experimental and clinical studies [17–22].

The aim of the study was to evaluate the influence of DFCS on the state of the malignant wound, including qualitative and quantitative features of its microbiota, intensity of wound odor and bleeding in Guerin's carcinoma-bearing rats with MFWs.

MATERIALS AND METHODS

Animals, tumor strain, sorbent. The study was performed on female Wistar rats weighing 270 ± 20 g from animal house of RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine in accordance with General Ethical Principles of Experiments on Animals (Ukraine, 2001) which are consistent with the provisions of "The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The Guerin T8 carcinoma cell strain was obtained from IEPOR Cell Bank of Human and Animal Tissue Lines. 0.5 ml of 20% carcinoma cell suspension in 0.9% sodium chloride solution was subcutaneously inoculated into the upper interscapular region of the back.

DFCS, based on activated carbon fibrous materials AUVM-“Dnepr” MN in 0.2% solution of zinc sulfate (Certificate № UA.TR. 101-100-2017) was used to treat MFWs.

Wound infecting and treating. *Staphylococcus aureus* 8325-4 strain from the collection of microorganisms of Department of Problems of Interferon and Immunomodulators, Zabolotny Institute of Microbi-

ology and Virology, NAS of Ukraine was chosen for modeling the infected wound due to two reasons: 1) among the ten bacteria most often identified in malignant wounds, more than 60% are *S. aureus* [13]; 2) *S. aureus* 8325-4 strain contains the plasmid of resistance to gentamicin and can be separated from other microorganisms in the growth medium containing this antibiotic (15 µg/ml). The strain was grown on BAIRD-PARKER-Agar (Merck, Germany) at 37 °C for 24 h. Daily culture of *S. aureus* 8325-4 was twice washed from agar medium with 0.15 M NaCl using centrifugation (3 000 rpm, 10 min) and then re-suspended in 0.15 M NaCl to obtain final concentration $1 \cdot 10^9$ colony forming units/ml (CFU/ml). On the 20th day after inoculation, surgical intervention for creating the small crater on the top of tumors was carried out. 4 h later, 1 ml of *S. aureus* 8325-4 suspension was administered into tumor.

The animals were randomly divided into two groups: control group ($n = 7$) — tumor volume 18.4 ± 3.8 cm³, wounds treatment with sterile gauze dressings; experimental group ($n = 7$) — tumor volume 19.5 ± 4.1 cm³, wounds treatment with sterile DFCS. Tumor volume was determined by three orthogonal tumor sizes (a, b and c) and was calculated by the formula: $V = (a \cdot b \cdot c) \cdot \pi/6$. After 24 h, gauze dressings and DFCS were applied to the pre-treated (hydrogen peroxide, antiseptic solution) wound surface, following a preliminary visual assessment of their state and taking the material from the contents of the wounds for microbiological analysis (initial value level — point “0”). To prevent dressings from drying the second layer (gauze, cotton wool) abundantly moistened with an antiseptic solution and waterproof layer (parchment paper or thin plastic film) were used. Animals were housed in individual cages throughout the experiment to prevent the dressing removal. From the beginning of treatment, the state of wounds was assessed *via* visual control with photo-monitoring, odor control according to the verbal rating scale from 1 to 4, where 1 — absent (no odor), 2 — minimal (barely noticeable), 3 — moderate (noticeable), and 4 — strong (intolerable) odor [11], and qualitative and quantitative microbiological control of wounds.

Microbiological analysis of wound contents.

Material was taken from the contents of the wound for microbiological analysis before dressing application, and on the 3rd, 6th, 10th and 16th day after their application. Material was collected using sterile cotton swab moistened with sterile saline. After collection, the swab was placed in a plastic tube into which 1 ml of sterile saline was added. Series of dilutions in normal saline to 10^{-3} and 10^{-5} were prepared from the initial dilution; 50 µl of each diluted sample was plated on Petri dishes with appropriate selective media for the cultivation and enumeration of different groups of microorganisms, namely: meat-peptone agar — medium for cultivation of aerobic and optional anaerobic microorganisms; Baird-Parker agar (Merck, Germany) — selective medium for *Staphylococci*; Baird-Parker agar with

gentamicin in concentration of 15 µg/ml — selective medium for isolation of *S. aureus* 8325-4 strain; KF-Streptococcus agar (Merck, Germany) — selective medium for streptococci; Endo agar (HiMedia, India) — selective medium for coliform bacteria; Sabouraud agar (HiMedia, India) — selective medium for microscopic fungi; Candida Medium (HiMedia, India) — selective medium for *Candida* spp., and Pseudomonas agar (HiMedia, India) — selective medium for *Pseudomonas* spp.

After 48 h of cultivation at 37 °C, the number of colonies per Petri dish was calculated, given that one such colony corresponds to one bacterium. The multiplicity of sample dilution was taken into account during the calculation. The result was expressed as lg of CFU per 1 ml. All biological material, as well as all the tools and laboratory utensils used during the microbiological analysis, were decontaminated by autoclaving.

Statistical analysis. All received digital data were processed using the computer program Epi Info (version 8.0) by the method of variation statistics, as well as the Excel program from the service package Microsoft Office-2007 and -2010. Numerical data

was presented in the form of arithmetic average and standard deviations ($M \pm SD$). Zero hypothesis for the comparison groups was verified using non-parametric Wilcoxon — Mann — Whitney (U) and Kolmogorov-Smirnov criteria. Differences between the groups were considered statistically significant at $p < 0.05$.

RESULTS

Before start of dressings, swelling of the wound edges, hemorrhages and capillary bleeding were observed in all animals (Fig. 1, a and 2, a). Microbiological analysis confirmed the presence of *S. aureus* 8325-4 strain in the wounds, the number of which had slight fluctuations in individual animals (Fig. 2, a) and did not differ ($p > 0.05$) from the total number of *Staphylococci* (Fig. 2, b). Streptococci, coliform bacteria and pseudomonads were not detected (Table).

A day later, in the majority of animals in control group the wounds were partially covered with a natural scab (Fig. 1, b), as well as in the experimental group treated with DFCS fibers, impregnated with blood and wound discharge, formed an “artificial” scab, completely covering the wounds (Fig. 1, 2, b).

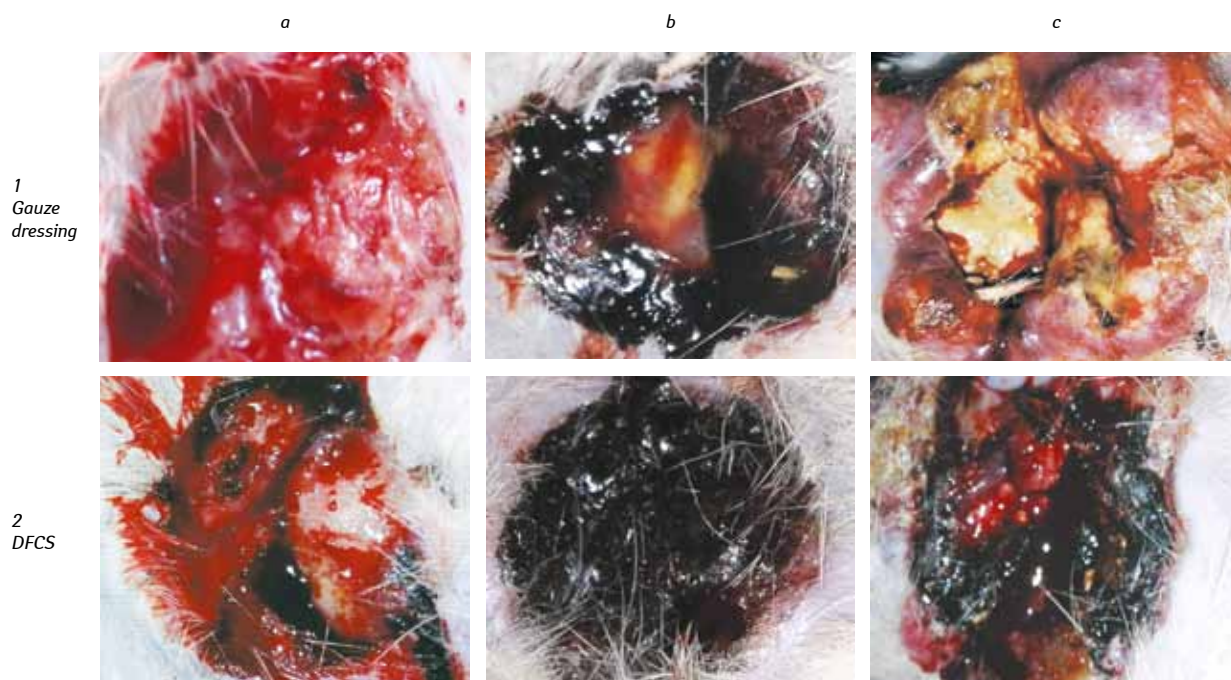


Fig. 1. The characteristic appearance of wounds in the control (1) and experimental (2) groups before the first dressing (a), on the next day (b) and the 10th day (c) after dressing

Table. Microbiota spectrum of wounds treated with gauze dressing and DFCS

Group of animals	Day	The number of microorganisms, lg CFU / ml, plated on selective nutrient media ($M \pm m$)					
		MPA	KF-Streptococcus agar	ENDO	Sabouraud agar	Candida medium	Pseudomonas agar
Gauze dressing (n = 7)	0	3.05 ± 0.09	0	0	1.17 ± 0.06	0	0
	3	8.61 ± 0.22	5.14 ± 0.21	0	0	0	0
	6	9.93 ± 0.10	2.42 ± 0.13	1.14 ± 0.13	2.44 ± 0.14	1.45 ± 0.06	1.93 ± 0.27
	10	9.05 ± 0.07	1.47 ± 0.17	1.56 ± 0.05	1.10 ± 0.07	0	2.17 ± 0.09
	16	9.45 ± 0.18	3.10 ± 0.22	1.18 ± 0.11	0	0	0
DFCS (n = 7)	0	2.77 ± 0.21	0	0	1.47 ± 0.13	0	0
	3	5.17 ± 0.19*	0*	0	0	0	0
	6	5.22 ± 0.14*	0*	0*	0*	0*	0*
	10	3.87 ± 0.08*	1.17 ± 0.18	1.13 ± 0.22	0*	0	0*
	16	3.10 ± 0.13*	0*	0*	0	0	0

Note: *the difference is significant compared with gauze dressing group ($p < 0.05$).

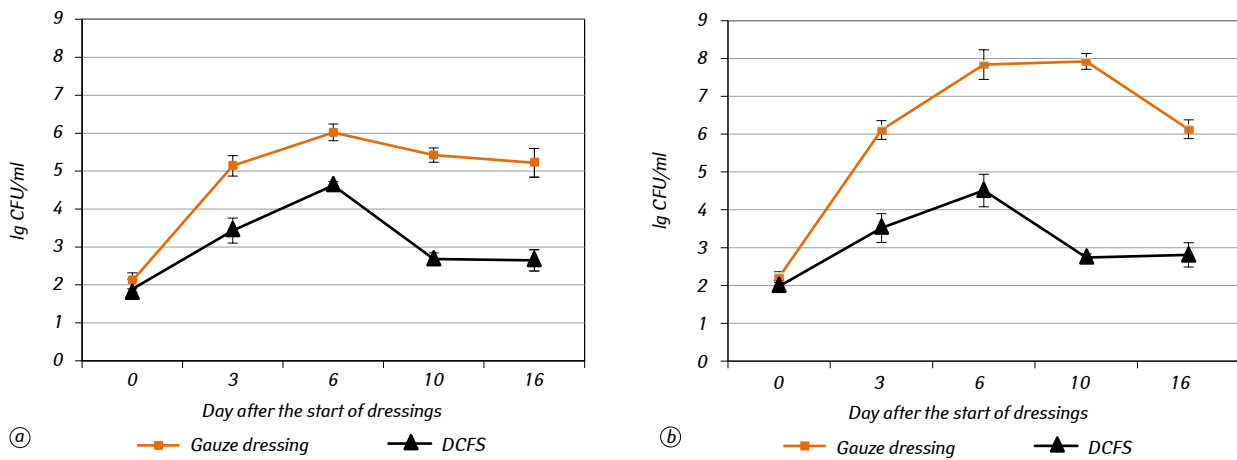


Fig. 2. The number of *S. aureus* 8325-4 (a) and the total number of *Staphylococci* (b)

On the 3rd day, in five control rats we observed moderate bleeding, and two animals developed an unpleasant odor corresponding to the definition of “barely noticeable”. The wounds in experimental animals mostly retained a carbon coating, slight capillary bleeding was observed in three rats, the odor was absent in all of them. By this day, the number of *S. aureus* 8325-4 strain has increased in the wounds of animals in both groups, although their number differed significantly in control and experimental ones being 5.14 ± 0.27 lg CFU/ml and 3.43 ± 0.33 lg CFU/ml correspondingly (Fig. 2, a). The total number of *Staphylococci* in the wounds of experimental rats did not differ statistically from the number of *S. aureus* 8325-4 strain while it was an order of magnitude higher in rats from the control group (Fig. 2, b). *Coliform bacteria*, microscopic fungi and *pseudomonads* were not detected in any group during this period (Table).

On days 6 to 10 after the start of dressings, the presence of purulent foci under gauze dressings and their absence, except one case, under carbon ones were recorded (Fig. 1, c and 2, c). In the control group, suppuration of wounds was accompanied by the odor enhancement from “noticeable” to “strong”. There was a transient appearance of a “barely noticeable” in one animal with a huge tumor ($V = 69.9 \text{ cm}^3$) in the experimental group. By day 10, three animals died in the control group and two in the experimental one.

The total number of *Staphylococci* as well as *S. aureus* 8325-4 in the gauze treated wounds remained consistently high, while in DFCS-treated wounds the total number of *Staphylococci* was almost 5 orders of magnitude lower and mainly represented by *S. aureus* 8325-4 strain (Fig. 2, a and 2, b). The total number of aerobic and facultatively anaerobic microorganisms was also significantly lower in the experimental group than in the control one (Table). Besides this, streptococci and coliform bacteria were still present in the wounds of control animals, the appearance of which in experimental rats was observed only on the 10th day. In the wounds of control group, microscopic fungi,

Candida spp. and *Pseudomonads* in some rats were detected.

On day 16, tissue necrosis was observed in rats of both groups. A difference in the state of wounds consisted of increased bleeding and the obligatory presence of odor in control rats, which was practically absent in experimental ones. By this day in each group one more animal died, the rest — over the next 7 and 10 days in control and experimental group relatively. On the 16th day, the total number of *Staphylococci* and *S. aureus* 8325-4 in the DFCS-treated wounds was almost equal to the initial values (point “0”), while in the wounds of the control rats these values were orders of magnitude higher: 3.10 ± 0.13 lg CFU/ml in DFCS-treated wounds against 9.45 ± 0.18 lg CFU/ml in the control ones (Fig. 2, a and 2, b). In addition, coliform bacteria and streptococci were present in the wounds of the control animals in an amount that was 1.5 orders of magnitude higher than on the 10th day, and were absent in the wounds covered with DFCS (Table).

DISCUSSION

The results obtained in the framework of the study allow us to highlight some facts indicating the effectiveness and feasibility for using the ADM in form of DFCS to treat the malignant wounds. This form of dressing was not chosen by chance. Dispersing carbon fibrous materials to individual fibers not only improves their adsorption-kinetic properties, but also guarantees more complete contact with the wound surface of any configuration.

Due to developed specific adsorption surface, reaching more than $2000 \text{ m}^2/\text{g}$, activated carbon fibrous materials has the high adsorption activity towards the substances of different molecular weight and nature. This is 3–10 times higher than that of activated carbon materials in dressings from well-known manufacturers Carboflex, Carbonet, and Actisorb Plus, which are today among commonly offered for controlling the malodorous wounds [23]. Due to unique adsorption-kinetic parameters ADM ensure rapid removal from the wound content of a large number of biologically active components, microbial cells,

bacterial endotoxins, decay products of necrotic tissues [17, 19]. One of the functional properties of ADM, noted by the vast majority of patients with wounds of different types, is the ability to reduce significantly wound pain [24].

In our experimental setting, the number of *S. aureus* 8325-4 as well as the number of other joined aerobic and optionally anaerobic microorganisms, which we fixed in rats under sterile gauze dressings, was significantly lower in DFCS — treated wounds. In addition, the total number of *Staphylococci* in the wounds of the control rats was significantly higher than the initial level of *S. aureus* 8325-4, while in the wounds covered with DFCS, it was not statistically different. Therefore, the presence of DFCS in the wound not only reduces the number of *S. aureus* 8325-4 via adsorption of microbial cells, but also prevents the accession of other species of *Staphylococci* to the infectious process. This also applies to bacterial contamination with other than *Staphylococci* microorganisms that were detected in the wounds of control rats and transiently in minimal amounts in DFCS-coated wounds. The ability of highly active carbon coating to provide a sorption barrier for external infection is of great importance for patients with malignant wounds, as well any other chronic wounds that require long-term treatment and long bedridden.

Qualitative and quantitative characteristics of the spectrum of the microbiota of wounds significantly influenced the odor of the wound and its intensity. A greater variety of microorganisms in the wounds of control rats, as well as an increase of their number were accompanied by enhancement of the wound odor from “noticeable” to “strong”, while in the experimental group a “barely noticeable” odor appeared temporarily in one animal only. The obtained result is based on the ability of carbon dressings to adsorb microbial cells and to bind volatile products of their vital activity, preventing their escape from the local wound area. In addition, carbon fibrous sorbents could become an adsorbing component of mask designed to protect against fetid odors and intended for both patients and sick-nurses.

To sum up, we demonstrated the ability of DFCS to control noticeably the bacterial microflora as well as malodor of malignant wounds, which were modeled in Guerin’s carcinoma-bearing rats. The results obtained could become the basis for innovative solutions to the problem of improving the quality of palliative care for patients with MFWs.

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ЕФЕКТИВНІСТЬ ДИСПЕРГОВАНОГО ВОЛОКНИСТОГО ВУГЛЕЦЕВОГО СОРБЕНТУ ПРИ ЛІКУВАННІ ЗЛОЯКІСНИХ ФУНГІДНИХ РАН

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Мета: Вивчити вплив диспергованих волокнистих вуглецевих сорбентів (ДВВС) на стан злоякісних фунгідних ран у щурів з карциномою Герена з оцінкою якісних та кількісних особливостей бактеріальної мікрофлори ран та її взаємозв'язку з інтенсивністю запаху. **Матеріали та методи:** Дослідження виконано на самках щурів лінії Вістар, яким в верхню міжлопаткову ділянку підшкірно інокулювали штам клітин карциноми Герена Т8. На 20-ту добу пухлини було інфіковано ін'єкцією суспензії *S. aureus* 8325-4. Че-

рез 24 год на рани в контрольній групі накладали марлеві пов'язки та ДВВС в експериментальній групі. Перев'язки проводили щодня. Стан ран оцінювали шляхом візуального контролю з фотомоніторингом, контролю запаху за вербальною рейтинговою шкалою та мікробіологічного аналізу якісних та кількісних характеристик мікробіоти рани. **Результати:** На 3-тю добу після початку перев'язок кількість стафілококів штаму *S. aureus* 8325-4 була значно більшою у ранах щурів контрольної групи, ніж експериментальної ($5,14 \pm 0,27$ проти $3,43 \pm 0,33$ lg CFU/ml). Загальна кількість стафілококів у ранах під ДВВС статистично не відрізнялася від кількості *S. aureus* 8325-4, тоді як під марлевими пов'язками була на порядок більшою. На 10-ту добу загальна кількість стафілококів та *S. aureus* 8325-4 штаму зокрема, в ранах контрольних щурів залишалася стабільно великою, тоді як у експериментальних тварин була майже на 5 порядків нижчою і головним чином представлена *S. aureus* 8325-4. Загальна кількість аеробних та факультативно анаеробних мікроорганізмів у тварин цієї групи також була значно меншою. Більша різноманітність мікроорганізмів у ранах контрольних щурів, а також збільшення їх кількості супроводжувалося посиленням запаху рани від "помітного" до "сильного". В експериментальній групі лише у однієї тварини спостерігалася тимчасова поява "ледве помітного" запаху. **Висновок:** Проведені дослідження продемонстрували здатність ДВВС помітно контролювати бактеріальну мікрофлору, а також неприємний запах злоякісних ран, які були змодельовані у щурів з карциномою Герена. Отримані результати можуть сприяти вирішенню проблеми підвищення якості паліативної допомоги хворим на злоякісні та інші хронічні рани.

Ключові слова: адсорбційні перев'язувальні засоби, вуглецеві пов'язки, злоякісні фунгідні рани, хронічні рани, мікробіота рани, запах рани.