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Features of healing of full-thickness skin wounds in laboratory rats under gauze and hydrogel dressings

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Abstract

Objective. To compare the dynamics and duration of healing of full-thickness skin wounds under gauze and hydrogel dressings (Vap-Gel and HydroTac Transparent), as well as to evaluate their effect on the activity of immunocompetent cells and histological features of skin regeneration.

Materials and methods. Thirty-one female Wistar rats were used, and full-thickness skin defects were created in the interscapular region. Three groups received different dressings: gauze (control), Vap-Gel, and HydroTac Transparent. Healing was monitored for 20 days through wound measurements, histological analysis, and immunological studies. The data has been processed statistically.

Results. It was found that the Vap-Gel hydrogel particles were integrated into the granulation tissue. Immunological studies highlighted reduced neutrophil activity in Vap-Gel-treated wounds. Both of these features had no significant effect on the duration of wound healing. HydroTac Transparent caused slower epidermization compared to gauze. Histological analysis revealed alterations in epidermis thickness and granulation tissue structure among groups. The mean epidermal thickness on the wound surface was 20-30 percent thinner in the groups treated with hydrogel dressings compared to the control group ($p < 0.05$).

Conclusion. It suggests that hydrogel dressings offer some advantages over traditional gauze in healing uninfected wounds. In general, the studied wound dressings provided a similar speed of the wound healing process. However, the observed changes in granulation tissue structure and local immune response suggest the need for further research to better understand the mechanisms of hydrogel dressings and optimize their application in wound care.

Keywords: *wound healing, hydrogel dressings, gauze dressings, full-thickness skin defects, histological analysis, immunological response*

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Особенности заживления полнослойных кожных ран у лабораторных крыс под марлевыми и гидрогелевыми покрытиями

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Резюме

Цель исследования. Сравнить динамику и продолжительность заживления полнослойных кожных ран под марлевыми и гидрогелевыми покрытиями (ВАП-гель и HydroTac Transparent), а также оценить влияние этих покрытий на активность иммунокомпетентных клеток и гистологические особенности строения кожного регенерата.

Материалы и методы. В межлопаточной области у 31 крысы-самки линии Wistar были нанесены полнослойные кожные раневые дефекты. Животные были разделены на три экспериментальные группы, для которых применялись разные перевязочные материалы: марля (группа № 1, контрольная) и два вида гидрогелевых покрытий (группы № 2 и № 3). За заживлением ран наблюдали в течение 20 дней. Проведен морфометрический анализ динамики контракции раны, иммунологические исследования и гистологический анализ кожи. Данные обработаны статистически.

Результаты. Установлено, что частицы гидрогеля ВАП-гель интегрировались в грануляционную ткань. Иммунологические исследования выявили снижение активности нейтрофилов в смывах с ран, на которые накладывался этот материал. Однако это не оказало существенного влияния на продолжительность заживления ран. Показано, что HydroTac Transparent приводил к более медленной эпидермизации ран по сравнению с марлевой повязкой. Гистологический анализ выявил различия в толщине эпидермиса и структуре грануляционной ткани между группами. Средняя толщина эпидермиса на раневой поверхности была на 20–30 % меньше в группах животных под гидрогелевыми повязками по сравнению с контрольной группой ($p < 0,05$).

Заключение. Считается, что гидрогелевые повязки обладают некоторыми преимуществами перед традиционной марлей при заживлении неинфицированных ран. В целом, исследованные гидрогелевые перевязочные материалы обеспечивали практически одинаковую скорость процесса ранозаживления в сравнении с марлевыми повязками. Однако наблюдаемые изменения в структуре грануляционной ткани и местном иммунном ответе указывают на необходимость дальнейших исследований для уточнения механизмов действия гидрогелевых перевязочных материалов и оптимизации их применения.

Ключевые слова: *заживление ран, гидрогелевые повязки, марлевые повязки, полнослойный кожный дефект, гистологический анализ, иммунологический ответ*

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Introduction

Injuries to human skin, resulting in wound formation, can arise from various causes, including mechanical, thermal, chemical, and trophic ulcers [1, 2]. These wounds are a frequent challenge in medical practice. Consequently, developing dressings that both isolate wounds from the external environment and create optimal healing conditions is a critical medical task [3, 4].

In recent years, hydrogel-based dressings have gained widespread use in medicine [5-8]. These dressings often surpass traditional gauze-based dressings in key aspects, such as extended application periods, easier removal, reduced patient pain, and the ability to incorporate healing-promoting medications [9] or antimicrobial agents [10].

Scientific advancements in dressing materials depend on laboratory models that can effectively demonstrate the properties of new materials compared to those already in medical use.

This study **aimed** to compare the wound healing properties of two hydrogel dressings available in Belarus with traditional gauze bandages.

Materials and methods

Thirty-one female Wistar rats, weighing 200–260 grams, were individually housed, provided with a standard diet, and given unrestricted water access. All procedures requiring anesthesia or immobilization, including stitching a protective chamber, creating full-thickness skin wounds, wound dressing, and euthanasia, were conducted under ether anesthesia following the guidelines of technical codes of established practice 125-2008 “Good Laboratory Practice” [1].

Full-thickness skin defects were created in the interscapular region as described earlier [11]. To do this, the fur on the interscapular region was manually removed, and the area was treated with 70 % ethanol. A round protective chamber was then sutured to the skin. A full-thickness wound was created by excising a 1 cm² area of skin, including the dermis and subcutaneous muscle, down to the subcutaneous tissue. The wound area varied among the animals, typically ranging from 110 to 140 mm² due to resulting skin stretching.

1.. TCP 125–2008 (02040) Good Laboratory Practice (GLP). – Ministry of Health of Republic of Belarus. – Minsk, 2008. – 35 p.

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Experimental Groups:

— Group 1 (control group) consisted of nine rats, with the wound area covered by discs (18 mm in diameter) made of bleached medical gauze "Blakit" (Baranovichi Cotton Production Inc, Belarus) soaked in 200 µl of saline;

— Group 2 contained eleven rats, with the wound area covered by discs made of Vap-Gel hydrogel dressings (Additional Liability Company "Radmedtech", Belarus);

— Group 3 consisted of eleven rats, with the wound area covered by discs made of HydroTac Transparent hydrogel dressings (Paul Hartmann AG, Germany).

All wound dressings were covered additionally with a gauze swab. The protective chamber was then capped with a 0.2 mm thick piece of aluminum foil.

For Group 1, gauze dressings were replaced daily until complete healing. In Groups 2 and 3, hydrogel dressings were replaced every three days until day 13. Thereafter, gauze dressings were used for all groups to facilitate epidermization assessment.

During dressing replacement, the wound surfaces were photographed using a Cybershot DSC W800 camera (Sony, Japan). Then, images were analyzed with ImageJ v. 1.54 software (National Institute of Health, USA) to measure the absolute wound area. Standardizing the initial wound area to 100 % minimized variability in statistical analysis due to size differences among animals.

On day 10, twelve rats (four per group) were euthanized for histological analysis. For histological examination of regenerates in the area of the former wounds two per group were euthanized 20 days post-operation. In both cases, rectangular-shape skin pieces, oriented along the sagittal line, were excised, extending to the subcutaneous tissue. Tissue samples were fixed in a formalin-alcohol-acetic acid solution, dehydrated, and embedded in paraffin wax.

Vertical sections (4.5 microns thick) were obtained using a rotary microtome Kedee 3398 (Zhejiang Jinhua Kedee, China), running along the sagittal line relative to the rat's body and spaced 750 microns apart. The sections were stained with hematoxylin and eosin. Slides were photographed using a Leica DM6-B microscope (Leica Microsystems GmbH, Germany) to obtain panoramic images (Figure 1).

Blood samples were collected from the tail vein immediately before wound creation and on day 20 to assess white blood cell count. Neutrophil phagocytic activity was evaluated using latex particle ingestion test as described everywhere. Additionally, neutrophil activity was analyzed in wound flushes collected on day 4 post-operation [12].

Statistical analysis of the data obtained was carried out with the support of Excel (Microsoft, USA) and Prism v.5 (GraphPad, USA) software. The normality of data distribution and homogeneity of the variances were assessed using the Shapiro-Wilk test. The statistical comparisons were performed by a one-way ANOVA followed by a Tukey's *post hoc* test in the case of a normal distribution of data and equality of sample variances, or otherwise the Kruskal-Wallis test followed by the Dunn's test for multiple comparisons. Differences were considered significant at $p < 0.05$. Quantitative data are presented as M ± m, where M represents the arithmetic mean and m represents the standard error of the mean.

Results

Two key indicators were used to assess dressing quality: the average duration of wound healing and its rate (Figure 2). The highest rates of regeneration were observed in animals of Group 2 compared to the control group. The duration of healing for a full-thickness skin defect was statistically longer by 2 days in rats of Group 3, where the hydrogel HydroTac Transparent was applied. This was attributed to the larger initial size of the full-thickness skin defect and the lower average healing rate observed in Group 3 (Figure 2).

Visual observation of the wound healing process revealed that it occurred similarly across all groups, characterized by a reduction in the total wound area (due to contraction) and an increase in epidermal growth from the edges of the intact skin to the wound surface. It is notable that, compared with gauze dressings, more fibrin accumulated on the wound surface under hydrogels, especially under the VAP-Gel.

An additional significant indicator for assessing the impact of the dressings on the healing process of a full-thickness skin defect is the dynamics of wound surface area reduction, measured both along the edge of the intact dermis and the edge of the epidermis (Figures 3-5). The rate of reduction in the size of the skin area devoid of dermis was greatest in animals of all three groups between days 4 and 10, while the rate of reduction in the area of the skin not covered with epidermis was greatest between days 7 and 13 (Figures 3, 4).

Ten days after the full-thickness skin defect was created, the wound area (measured along the edge of the intact dermis) in the Vap-Gel-treated group (Group 2) was significantly smaller than in the control group. In contrast, by day 16, the wound area still lacking epidermal coverage in the HydroTac Transparent group (Group 3) was significantly larger than that of the control group. This indicates that at later stages of regeneration, the HydroTac Transparent group experienced slower epidermization compared to the control group.

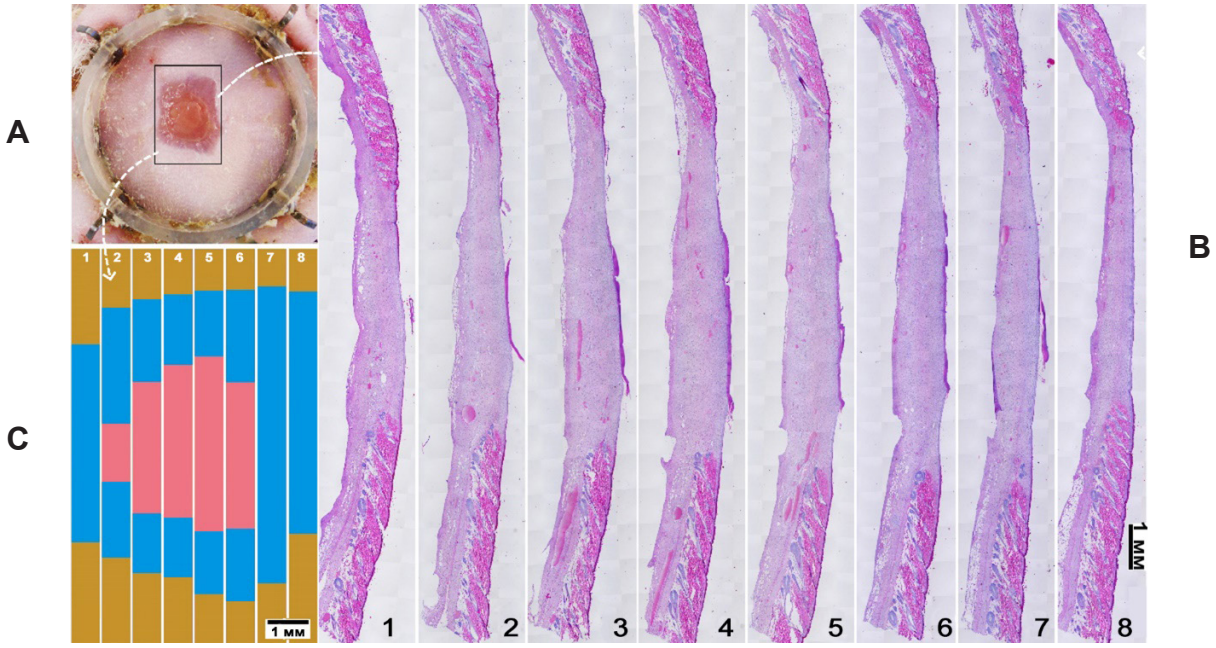

Figure 1. Histological sections from a skin area obtained from a full-layered skin defect and a 2D map based on them. A — macroscopic image of a full-thickness skin defect in one of the rats, taken 10 days post-creation. The histologically examined skin area is marked by a black frame. B — panoramic photographs of sagittal histological sections, obtained from the wound area at intervals of 750 μm and numbered 1 to 8. Hematoxylin-eosin staining. C — 2D-map illustrating the location of the main structures within the sections. Brown color indicates the presence of dermis covered with epidermis, blue signifies the presence of epidermis on the surface of granulation tissue, and pink represents granulation tissue without epidermis

Рисунок 1. Гистологические срезы участка кожи, полученного из полнослойного кожного дефекта, и созданная на их основе 2D-карта: А — макроскопический вид полнослойного кожного дефекта у одной из крыс через 10 суток после нанесения. Участок, исследованный гистологически, обведен черной рамкой; В — панорамные фотографии сагиттальных гистологических срезов, полученных из области раны с интервалом 750 мкм, пронумерованных от 1 до 8; С — 2D-карта расположения основных структур на срезах. Коричневый цвет соответствует присутствию дермы, покрытой эпидермисом; голубой соответствует наличию эпидермиса на поверхности грануляционной ткани; розовый обозначает грануляционную ткань без эпидермиса

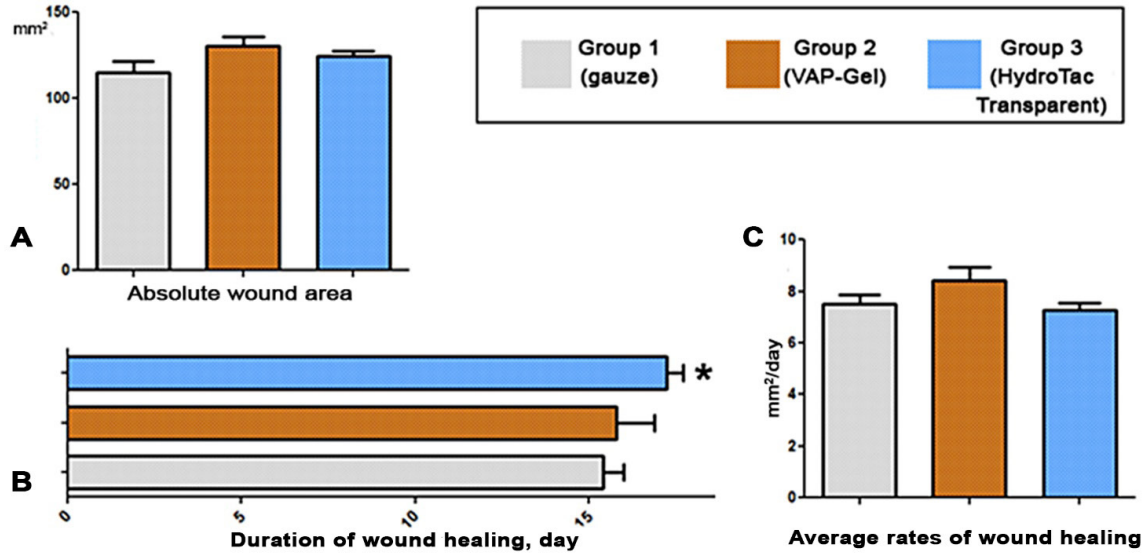

Figure 2. Initial sizes of full-thickness skin defects, duration and rate of healing in three groups of rats: A — Initial dimensions of full-thickness skin defects, mm2; B — The duration of wound healing between groups, days; C — Average rates of wound healing, mm²/day.
** — p <0,05*

Рисунок 2. Исходные размеры полнослойного кожного дефекта, продолжительность и темпы заживления в трех группах крыс: А — исходный размер полнослойного кожного дефекта, мм²; В — продолжительность заживления ран между группами, дней; С — средняя скорость заживления раны, мм²/сут.
** – p < 0,05.*

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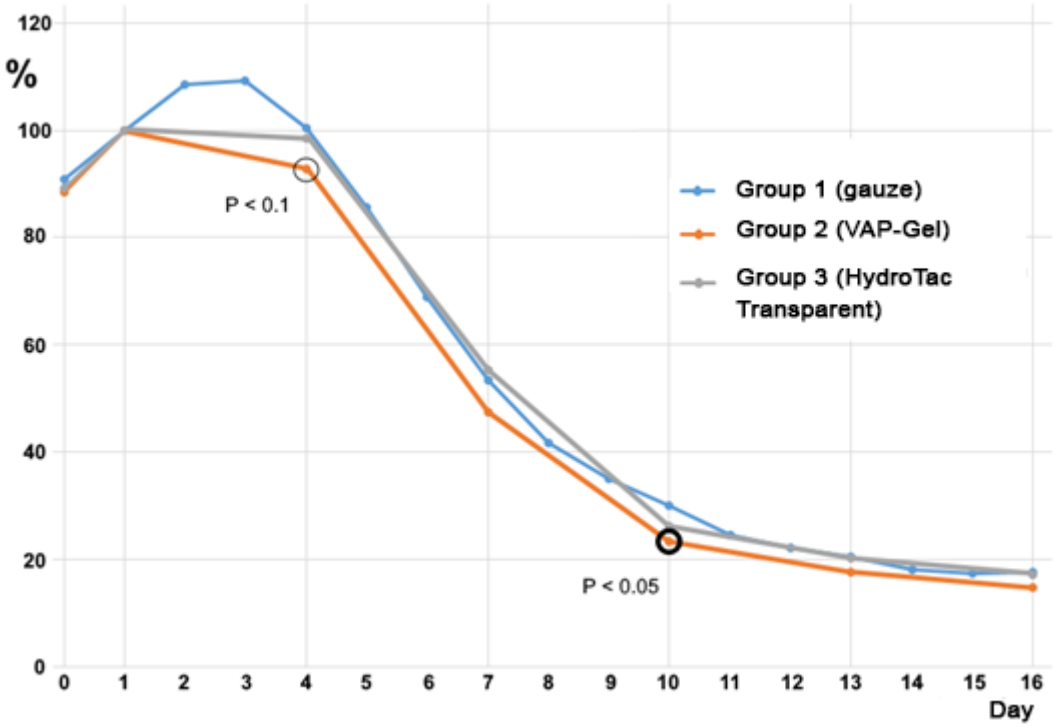

Figure 3. Changes in the area of the wound surface under different dressings, which was measured along the edge of the intact dermis. The dynamics of the contraction process in animals of the 2nd and 3rd groups are close to those of the animals of the control (group 1)

Рисунок 3. Изменение площади раневой поверхности под различными покрытиями, которая была измерена по краю интактной дермы. Динамика процесса контракции у животных 2-й и 3-й групп близка к животным 1-й (контрольной) группы

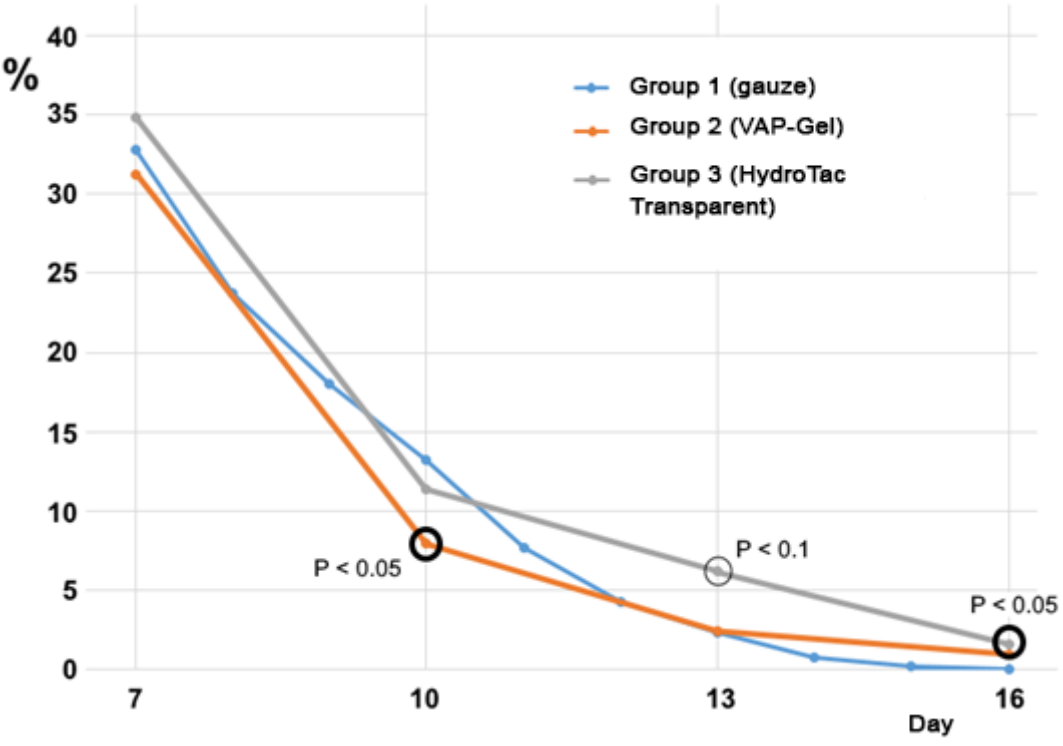

Figure 4. Dynamics of the wound area measured along the edge of the epidermis, which grew on the wound surface under different dressings

Рисунок 4. Динамика изменения площади раны, замеренной по краю эпидермиса, который нарастал на рану под различными раневыми покрытиями

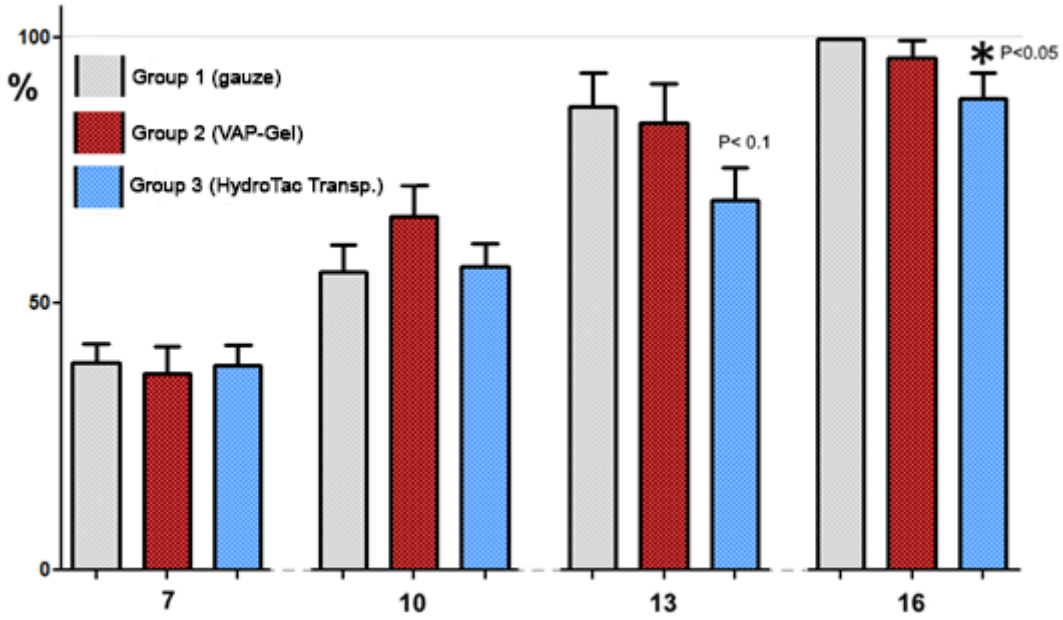

Figure 5. The degree of epidermization of the wound (in % relative to its area at the corresponding moment) at the final stage of regeneration. There is a certain inhibition of the epidermization of the wound surface in animals of 3rd group compared to the control
Рисунок 5. Степень эпидермизации раны (в % относительно ее площади в соответствующее время) на заключительном этапе регенерации. Наблюдается определенное торможение эпидермизации раны у животных 3-й группы по сравнению с контролем

During histological examination, additional phenomena were observed. In the control group, where the wound surface healed for 10 days under a gauze dressings soaked in saline solution developed granulation tissue was present. This tissue was filled with collagen fibers, fibroblasts, macrophages, blood vessels, adipocytes, mast cells, neutrophil, and eosinophil leukocytes at the site of the former defect. A thin layer of fibrin was observed on the wound surface, typically containing neutrophilic granulocytes and macrophages. The epidermis grew from the edges of the intact skin, moving along the surface of the granulation tissue, and included basal, spinous, and granular layers. Approximately 1 mm from the anterior edge, a thin stratum corneum was already present on the epidermis.

In the granulation tissue of the control group, thin fibrils (gauze) were found embedded in the granulation tissue, surrounded by macrophages that formed foreign-body giant cells.

In Group 2, where rats were treated with Vap-Gel for 9 days, the overall appearance of the wound tissue was similar to that of other groups. However, histological analysis revealed that some gel particles had penetrated into the granulation tissue. This was further confirmed by examining tissue samples collected after 20 days (Figure 6). Importantly, the presence of Vap-Gel particles in the granulation tissue did not hinder the rate of healing or the process of wound contraction.

In 3rd group of rats, the pattern of regeneration was similar to that observed in the first two groups. No foreign-body giant cells were found in the granulation tissue, but the epidermis that grew on the wound surface was thinner compared to the control group.

The measurements of epidermis thickness on the wound surface after 10 days of healing are presented in Table 1. In Groups 2 and 3, where hydrogel dressings were used, the epidermis was noticeably thinner compared to the control group. This difference may be due to environmental factors affecting the healing process with hydrogel dressings, such as higher humidity levels and reduced exposure to atmospheric oxygen [13, 14].

Neutrophil saturation of the surface layer of granulation tissue was similar in all three groups. However, the degree of blood vessel filling (mainly venules) in the granulation tissue of Groups 2 and 3 was greater than in the control group. In animals of all three groups, mast cells, macrophages, whose cytoplasm was filled with lipofuscin, and eosinophils were found in deeper layers of granulation tissue. The largest number of the latter cellular forms could be found in some animals of the 3rd group.

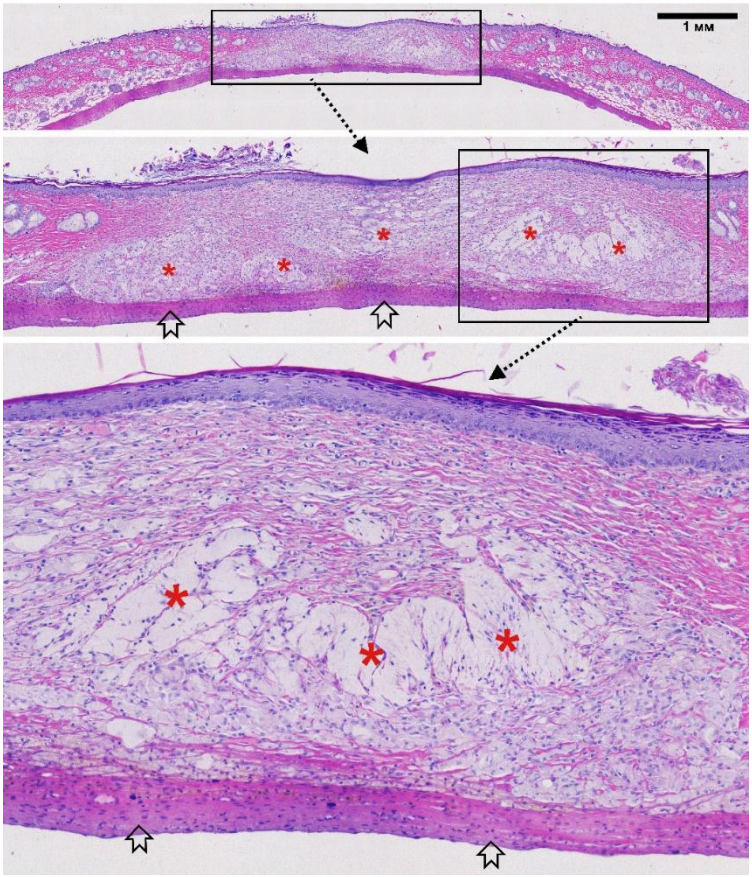

Figure 6. Histological structure of the regenerated tissue 20 days after creating a full-thickness skin wound in a rat from the 2nd group (treated with Vap-Gel).
The regenerate is completely covered with epidermis with a stratum corneum. On the sides of it are the edges of the intact dermis, in which the intradermal parts of the epidermal derivatives are visible (top photographs). A significant volume of granulation tissue consists of WAP-gel particles that have penetrated from the surface of the wound into its depths (marked with red asterisks). Black arrows point to the newly formed elastic layer under the granulation tissue
Рисунок 6. Гистологическая структура регенерата на месте полнослойного кожного дефекта через 20 суток после его нанесения у одной из крыс 2-й группы.
Регенерат полностью покрыт эпидермисом с роговым слоем. По бокам от него находятся края интактной дермы, в которой видны интрадермальные части производных эпидермиса (вверху). Значительный объем грануляционной ткани составляют частицы ВАП-геля, которые с поверхности раны проникли вглубь (обозначены красными звездочками). Черными стрелками обозначена эластичная прослойка, новообразованная под грануляционной тканью

Table 1. Thickness of the epidermis present on the wound 10 days after the creation of a full-thickness skin wound, μm
Таблица 1. Толщина эпидермиса на ране через 10 суток после создания кожного дефекта, мкм

	At the front edge of the wound	On the posterior edge of the wound	On both wound edges together
	1st Group, Control		
M ± м	66.25±8.94	59.5±4.21	61.7±5.18
	2nd Group, VAP-Gel		
M ± м	51.6±3.51	46.3±3.81	48.0±2.05
p	0.1785	0.0586	0.0495
	3rd Group, HydroTac Transparent		
M ± м	40.2±5.48	46.6±7.33	42.2±6.00
p	0.0475	0.1765	0.0489

The impact of the wound healing process and dressing materials on the rats' overall health was assessed using an immunological study. Before the full-thickness skin defect was created, the white blood cells count of all experimental groups were nearly identical. However, 20 days after the procedure, all rats showed an increase in segmented neutrophils and a decrease in lymphocytes in their blood. Furthermore, rats in the 3rd group (treated with HydroTac Transparent) had a lower proportion of eosinophils compared to the control group.

There were no statistically significant changes in the phagocytic index of blood neutrophils 20 days after the creation of the full-thickness skin defect compared to the initial state. The same applied to the NBT test scores.

An evaluation of neutrophilic leukocyte activity in wound surface flushes, conducted 4 days post-operation, showed a reduced phagocytic index in 2nd group of animals (Table 2). Despite this reduction and the previously observed Vap-Gel penetration into the wound, no adverse effects were noted on wound contraction or epidermization. This indicates that neutrophilic leukocytes, while important for controlling wound microflora, may play a less critical role than other cell types in the healing of full-thickness skin defects when pathogenic microorganisms are absent.

Table 2. Indicators of phagocytic activity of neutrophils in wound surface flushes, taken 4 days after the creation of a full-thickness skin wound
Таблица 2. Показатели фагоцитарной активности нейтрофилов в смывах с раневой поверхности, взятых через 4 дня после нанесения кожного дефекта

Groups	Phagocytic index (with adhered particles), %	Phagocytic index (without adhered particles), %
1st Group, Control	72.25±3.56	55.6±5.24
2nd Group, VAP-Gel	59.9±2.92 p_{1-2}=0.0155	36.2±6.89 p_{1-2}=0.0553
3rd Group, HydroTac Transparent	82.6±3.15 p_{1-3}=0.0445 p_{2-3}< 0.0001	64.8±2.87 p_{1-3}=0.1622 p_{2-3}< 0.0050

A correlation analysis was performed to identify relationships between the quantitative parameters obtained from the measurements. Among the most significant indicators characterizing the wound healing process, the following correlations were observed: a strong positive correlation between the duration of healing of a full-thickness skin defect and the degree of its epidermization (estimated at 13th day of healing, r = 0.78; p = 0.0010), and a moderate negative correlation between the rate of wound healing and the total duration of this process (r = –0.53; p = –0.0511).

The duration of skin regeneration was also positively associated with the relative content of segmented neutrophils in the blood taken 20 days post-surgery (r = 0.56; p = 0.0384), and negatively associated with the relative content of lymphocytes in the same samples (r = –0.67; p = 0.0094).

These findings demonstrate that the wound healing process is not merely a local phenomenon but rather a systemic process involving the entire organism.

Conclusion

A study conducted on 31 laboratory rats, in which a full-thickness skin defect was simulated in the interscapular region, aimed to identify the effects of three types of dressings on the healing process. Despite the varying conditions created by these dressings on the wound surface, they generally ensured a primarily similar course of wound healing. Rats in all groups exhibited consistent weight gain during the first 20 days of the study. Granulation tissue successfully formed at the site of the wound, and the skin defect was reduced in size by contraction and simultaneously epidermization due to the growth of the epidermis from the adjacent intact skin.

A more detailed analysis revealed specific features of wound healing in the different groups. In the control group, gauze threads and thin fibers from these threads could become embedded in the granulation tissue. In the 2nd group (VAP-Gel), hydrogel particles demonstrated the ability to incorporate to the granulation tissue. This phenomenon requires further study, given that the 20-day follow-up period used in the study may not fully reflect the long-term effects of using these dressings, especially for chronic wounds. Although neutrophil granulocytes on the wound surface exhibited reduced phagocytic activity, this did not significantly affect the overall wound healing rate. In the 3rd group (HydroTac Transparent), a certain slowdown in the rate of regeneration was observed, likely due to the less active growth of the epidermis under the wound surface.

It suggests that hydrogel dressings offer some advantages over traditional gauze in healing uninfected wounds. However, the observed changes in granulation tissue structure and local immune response suggest the need for further research to better understand the mechanisms of hydrogel dressings and optimize their application in medical care. Overall, the data obtained can serve as a basis for further improvement of dressings designed to provide optimal conditions for wound healing.

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