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Experimental modelling of full-thickness skin wounds in pigs

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Abstract. In the modern context, there is a growing need to develop a relevant experimental model of a skin wound that closely replicates the regeneration processes occurring in human wounds. The aim of this study was to develop a method to prevent premature contraction of wound edges during the experimental modelling of a full-thickness skin wound in pigs, thereby creating optimal conditions for evaluating the effectiveness of local treatment approaches. An experimental study was conducted on a white pig weighing 15 kg. A full-thickness skin wound measuring 5×5 cm was created on the animal's back under thiopental sodium anaesthesia at a dosage of 80 mg/kg. Tissue samples were collected from the wound site via punch biopsy under general anaesthesia, fixed in 10% neutral formalin, and embedded in paraffin using standard histological techniques. Deparaffinised sections were stained with haematoxylin and eosin. A computer program was developed in Python to calculate the wound area using the Monte Carlo method. To visualise the results and observe trends, graphical representations in the form of diagrams were used. The study demonstrated the feasibility of modulating contraction in full-thickness skin defects by applying incisions. The most effective method involved tangential incisions at each corner of the wound, each measuring up to 1 cm in length. This technique reduced the degree of wound edge contraction. On day 28 of observation, the wound area in the experimental group was 69.3% of the original size, compared to 39.3% in the control group. To accurately assess the effectiveness of treatments for full-thickness skin wounds in porcine models, it is essential to maintain a wound of appropriate size for at least 28 days to allow for observation of scar tissue formation. The proposed wound model enables controlled modulation of contraction and preserves an adequate wound surface area for the duration necessary to study scar formation processes

Keywords: excisional wound model; porcine model; wound edge contraction; wound area; wound healing; dermal matrix

INTRODUCTION

The repair of full-thickness skin wounds is governed by complex mechanisms of tissue regeneration and repair. Novel therapeutic agents are continually being developed, particularly those targeting the restoration of the damaged dermal matrix. To evaluate the effectiveness of such treatments, various animal models are used that aim to replicate the characteristics of human skin wounds. However, the skin of small laboratory animals – such as rodents and rabbits – differs significantly from human skin, with wound healing in these species primarily occurring through contraction rather than re-epithelialisation. Additionally, their limited skin surface area often necessitates the use of a large number of animals to obtain sufficient experimental material, which can increase variability and reduce the reliability of results.

In contrast, pig skin closely resembles human skin in both structure and function. It features a thick epidermis, dense collagen and elastic fibres in the dermis, and the presence of hair and sweat glands. D.W. Hamilton *et al.* [1] emphasise that pigs and humans share similar physiological and anatomical characteristics, resulting in comparable mechanisms of wound healing. However, the authors also highlight the need for further research to establish pigs as a reliable model for studying human wound healing processes. Moreover, T. Ryk [2] suggests that pigs hold promise as potential organ donors for humans, further underscoring their biomedical relevance.

J. Shiff *et al.* [3] also support the use of the porcine wound model, arguing that it more accurately reflects the processes of wound repair in humans compared to rodent

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models. M. Elloso *et al*. [4] highlight the advantages of the porcine model for advancing the understanding of wound biology and developing new therapeutic strategies. This model plays a crucial role in preclinical research by effectively bridging the gap between *in vitro* studies and clinical trials. Additionally, M. Tucci *et al*. [5] point out that the body size of pigs offers sufficient skin surface to allow for the creation of multiple wounds on a single animal. This minimises the influence of extraneous factors and reduces inter-subject variability.

According to R.B. Diller & A.J. Tabor [6], one of the key factors in wound regeneration is the condition of the extracellular matrix, which is actively involved in all phases of the healing process. Restoration of dermal integrity through the regeneration of the dermal matrix can significantly improve treatment outcomes by promoting the formation of a structured and elastic scar with tensile strength and elasticity comparable to those of intact dermis. In cases where the dermis is damaged, E.M. Tottoli *et al*. [7] argue that advanced technologies are required, as routine clinical methods of local wound treatment are often insufficient to address the complexity of full-thickness skin defects effectively.

P. Bargavi *et al*. [8] note that when the microarchitecture of a bioengineered product closely mimics the natural extracellular matrix (ECM), it can effectively promote cell growth, ECM deposition, and the formation of new tissue. Similarly, J. Xu *et al*. [9] provide evidence that ECM substitutes create a microenvironment resembling native epidermal or dermal tissue, thereby supporting cell migration, angiogenesis, proliferation, differentiation, and ECM production during the wound healing process. C. Dai *et al*. [10] describe various commercially available skin substitutes designed to replace the ECM, derived from autologous, allogeneic, or xenogeneic sources.

To evaluate the efficacy of dermal substitutes, full-thickness wound models are commonly employed. The fundamental principles for creating such models are outlined in the work of T.Y. Kuo *et al*. [11], who provide recommendations regarding the optimal wound size, location on the pig's body, and the advisable number of wounds. Adhering to these recommendations is essential to avoid negatively impacting the wound healing process. For instance, placing wounds too close to one another may impair local blood circulation, while increasing the number and total area of wounds can induce systemic effects in the animal. These systemic changes may lead to infectious complications and can compromise the validity of experimental data by influencing the effectiveness of local treatment under conditions of general physiological stress in the animal.

Since one of the primary criteria for evaluating local treatment is the rate of wound area reduction, it is essential to understand the underlying mechanisms driving this process in experimental models. However, contemporary literature pays insufficient attention to the role of wound edge contraction in area reduction, which may lead to misinterpretation of results when assessing the effectiveness of local treatment strategies in wound repair. Given this issue, and the lack of solutions proposed in the current literature to address it, the author aimed to investigate methods for minimising the influence of wound edge contraction on granulation tissue formation. The objective was to establish a more relevant experimental model of a full-thickness skin wound that would allow for accurate assessment of the effectiveness of a dermal matrix substitute derived from porcine dermis, as a promising method for restoring the native extracellular matrix in damaged dermal tissue.

MATERIALS AND METHODS

A white pig weighing 15 kg was used for the experimental study. The animal was housed in the vivarium of I.Ya. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, and maintained on a standard diet in accordance with sanitary-hygienic regulations and Good Laboratory Practice (GLP) standards [12]. The surgical intervention was conducted during morning hours between March and May 2024, in full compliance with the general principles and provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [13], the Bioethics Guidelines [14], and the Law of Ukraine No. 3447-IV [15]. The Bioethics Commission of I.Ya. Horbachevsky Ternopil National Medical University confirmed that no violations of ethical standards occurred during the study (Protocol No. 80, dated 10 January 2025).

A full-thickness skin wound measuring 5×5 cm was created on the pig's back by excising a skin flap along with subcutaneous fat down to the superficial fascia, under thiopental sodium anaesthesia at a dosage of 80 mg/kg. The dynamics of wound healing were monitored over time without any intervention at the wound edges. Wound area measurements were taken on days 1, 7, 14, and 28 of the experiment. The wounds were divided into two groups: Group I received only aseptic dressings, while Group II was treated with an acellular dermal matrix derived from porcine skin. To investigate the effect of mechanical manipulation of wound edges on wound contraction, the wound variations illustrated in Figure 1 were created on the pig's back.

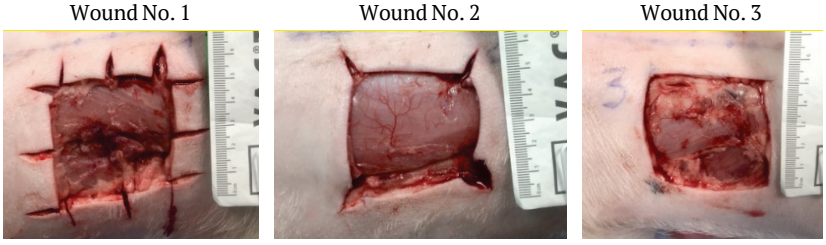

Figure 1. Variants of forming full-thickness wounds
Notes: wound No. 1: 5×5 cm, 1 cm incisions were made perpendicular to the wound edge; wound No. 2: 5×5 cm with linear incisions at the corners of the wound 1 cm long; wound No. 3: 5×5 cm, without affecting the wound edges (control group)
Source: created by the author

To assess the degree of structural changes within the wound, a morphological study was conducted. On the 28th day of the experiment, under general anaesthesia induced by sodium thiopental, wound tissue samples were collected using a 6 mm diameter punch biopsy. The specimens were fixed in 10% neutral formalin solution and embedded in paraffin blocks. Cross-sections 5-6 μm thick were prepared using a microtome. The resulting histological sections were stained with haematoxylin and eosin for microscopic examination (eyepiece 10×, objective 20×). To calculate the wound area, the Monte Carlo method was employed [16]. For this purpose, a custom computer program was developed using the Python programming language. The program is based on an algorithm comprising the following stages:

- Selection of a bounding figure: A simple geometric shape (e.g., a square or rectangle) that completely encloses the wound area is selected. The area of this shape can be calculated using standard geometric formulas.
- Generation of random points: A large number of random points are generated within the bounding figure. The coordinates of these points are determined using random number generators.
- Determination of point inclusion: Each randomly generated point is evaluated to determine whether it lies within the wound boundary. This is achieved by analysing the coordinates of the point in relation to the mathematical description of the wound's perimeter.
- Calculation of point fraction: The proportion of points falling within the wound area is calculated relative to the total number of generated points.
- Estimation of wound area: The wound area is then estimated by multiplying the area of the bounding figure by the fraction of points that lie within the wound boundary (1).

$$S_{figure} = S_{bounding\ figure} \cdot \frac{N_{successful}}{N_{total}}, \quad (1)$$

where S_{figure} – the area of the wound under study; $S_{bounding\ figure}$ – the area of the bounding figure; $N_{successful}$ – the number of points falling within the wound area; N_{total} – the total number of generated points.

The use of this algorithm enables accurate estimation of wound area, even for irregularly shaped surfaces, thereby providing an objective assessment of wound size at various stages of treatment. The image resolution used was 37 pixels/cm. The bounding figure was defined as a square with a side length of 6 cm. The number of pixels (points) within the bounding figure was calculated as the product of the area of the bounding figure and the number of pixels per cm² (2):

$$N = 6^2 \cdot 37^2 = 49{,}284. \quad (2)$$

To analyse trends in the change of wound surface area across the observation groups, a graphical method was employed using diagrams to visually represent the data.

RESULTS AND DISCUSSION

Based on formulas (1) and (2), the program generated a total of 49,284 random points. Each point was evaluated to determine whether it fell within the wound image or outside of it, in accordance with the aforementioned formulas. An example of the program's execution and output is presented in Figure 2.

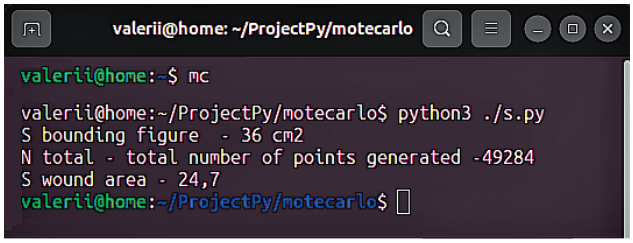

Figure 2. The result of the program for calculating the wound area using the Monte Carlo method
Source: created by the author

During the study, the dynamics of wound area reduction were monitored in two observation groups: Group I consisted of wounds closed without the use of a dermal matrix substitute, while Group II involved closure with an acellular dermal matrix derived from porcine skin (Fig. 3).

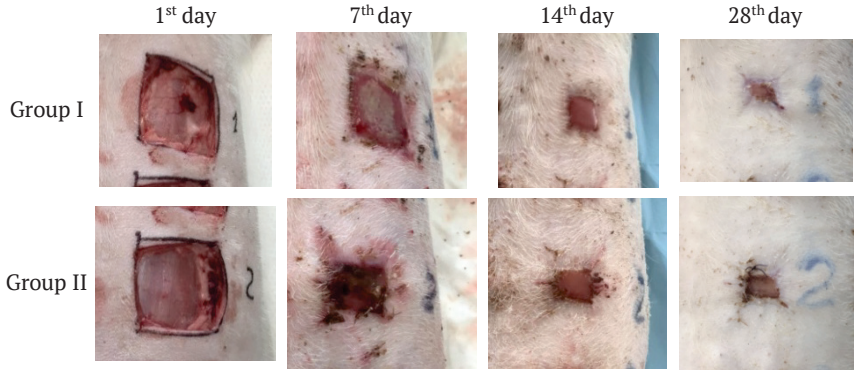

Figure 3. Dynamics of change in the area of the full-thickness wound of the first and second groups
Source: created by the author

During the observation period from day 1 to day 28, it was noted that the wound areas in both groups decreased at a similar rate. Visual assessment confirmed that the wound areas were so closely aligned that precise digital measurement was deemed unnecessary, as measurement accuracy in this context was not considered critical. By day 28, there was virtually no difference in wound area between the two observation groups. Of particular note, a marked reduction in wound area in both groups was already apparent by day 7, which is consistent with the findings of R. Elia *et al.* [17].

Under such conditions, it becomes impractical to evaluate the therapeutic effectiveness of the acellular dermal matrix in treating full-thickness skin defects. Figure 4 presents the histological findings from a biopsy taken from a Group I wound, while Figure 5 shows the histological result from a Group II wound on day 28 of the experiment.

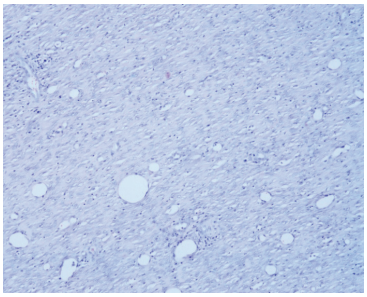

Figure 4. Biopsy from a wound of Group I
Source: created by the author

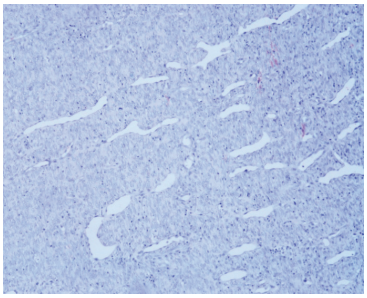

Figure 5. Biopsy from a wound of Group II
Source: created by the author

Both histological preparations revealed young connective tissue characterised by a high density of fibroblasts and focal lymphomacrophage infiltration. The connective tissue was well vascularised, with newly formed blood vessels visible. These findings suggest that the reduction in wound area was not primarily due to re-epithelialisation over newly formed granulation tissue, but rather due to contraction of the wound edges. The absence of a significant difference in granulation tissue formation between the two observation groups supports the hypothesis that an experimental model of a full-thickness wound in pig skin must meet specific temporal criteria. As noted by V. Coger *et al*. [18], the regeneration of full-thickness skin defects follows defined time intervals. Therefore, for such a model to remain relevant, it is essential to preserve a sufficient wound area for a certain duration – ensuring that closure does not occur predominantly through wound edge contraction. S.O. Udegbunam *et al*. [19] also emphasise the influence of wound edge contraction on the restoration of skin integrity. The strong tendency of full-thickness skin defects to contract is further highlighted by H.A. Wallace *et al*. [20], who reported that wound contraction contributed to 88% of closure, while scar formation accounted for only 12% of the wound surface.

A detailed analysis of the wound contraction mechanism is presented by S.E. Cross *et al*. [21], who demonstrated that excision of granulation tissue within the wound bed had no effect on contraction. Similarly, cutting the wound edges down to the deep fascia did not prevent contraction; on the contrary, it led to rapid adhesion of the wound edges to the wound bed, followed by resumed inward migration. The study showed that granulation tissue formed in the wound centre does not significantly contribute to contraction. Instead, a narrow rim of newly proliferated fibroblasts, located beneath the wound margin and measuring 1-2 mm in width, is responsible for wound closure. This mass of fibroblasts forms a subcutaneous “picture frame”, anchoring the dermal edges to the underlying deep fascia and pulling the intact dermis inward through directed collective migration.

It is important to note that in several classical excisional wound models – particularly in mice, as demonstrated by D.S. Masson-Meyers *et al*. [22] – healing occurs predominantly through contraction, which constitutes a significant component of the wound closure process. Traditionally, it was believed that complete wound closure by contraction was characteristic only of animals with highly mobile skin, such as guinea pigs, rabbits, and rats. However, the present findings clearly indicate that even animals with relatively immobile skin, such as pigs, also experience substantial contraction, which can result in a marked reduction of the wound area. In the course of the conducted observations on full-thickness defect closure in pig skin, it was found that the contraction of wound edges in pigs was sufficiently pronounced to draw the surrounding skin inward, thereby significantly contributing to the closure of the wound defect.

S.A. Park *et al*. [23] and X. Wang *et al*. [24] addressed a similar issue in rodents by developing a splinted wound model to suppress dermal contraction and improve the translational relevance of the mouse model for studying human wound healing. This technique involves the creation of full-thickness wounds on the dorsal surface of the mouse, followed by the placement of a silicone splint at the wound site, which is then secured to the skin with sutures to prevent contraction. Consequently, wound healing in this model proceeds via granulation tissue formation and re-epithelialisation, closely mimicking the human healing process. However, no studies have been reported in the literature describing the application of similar mechanical interventions to prevent wound contraction in porcine models. This highlights a significant gap and the need for further research to develop and validate methods that can suppress contraction in pigs, thereby increasing the relevance of this model for studying full-thickness wound healing in humans.

According to the hypothesis proposed by the author of this study, mechanical damage to the wound edges would result in disruption of the collagen fibres to which myofibroblasts are attached. It was anticipated that such disruption would weaken the contractile forces responsible for wound narrowing and thereby slow the reduction of wound area. This hypothesis was informed by the findings of S.M. Karppinen *et al*. [25] and F. Chang *et al*. [26], who reported that myofibroblasts play a central role in wound contraction. These cells exert contractile forces by attaching to the extracellular matrix via integrins and generating tension through stress fibres rich in alpha-smooth muscle actin.

Figure 6 illustrates the dynamics of changes in wound surface area during the observation period following mechanical disruption of the wound edges in wound No. 1 and wound No. 2. Wound No. 3 served as the control. In determining the number and orientation of incisions, the author followed several guiding principles: to ensure that the additional trauma to the wound edges did not increase the risk of infection; to preserve the overall wound contour, thereby enabling accurate measurement of the wound area throughout the observation period; and to retain the possibility of fixing a bioengineered product to the wound edges for improved attachment if necessary.

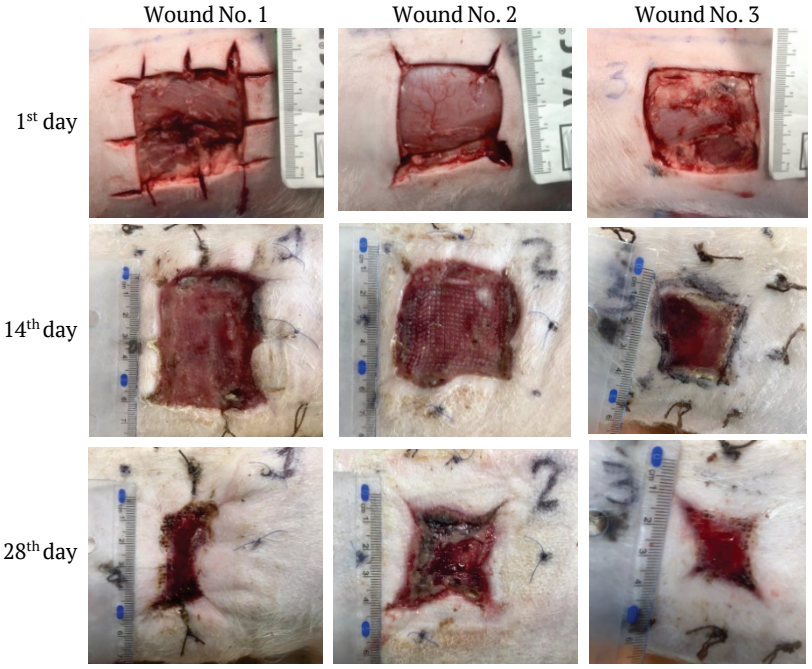

Figure 6. Dynamics of change in wound area

Source: created by the author

The application of different methods of mechanical intervention on the wound edges resulted in varying effects, as evidenced by differing degrees of wound area reduction over the observation period. The use of the Monte Carlo method enabled accurate and efficient calculation of wound surface area, even for irregularly shaped wounds. The results of wound area measurements obtained using this method are presented in Table 1.

Table 1. Wound area in sq. cm

	Wound No. 1		Wound No. 2		Wound No. 3	
	$N_{successful}$	S_{figure}	$N_{successful}$	S_{figure}	$N_{successful}$	S_{figure}
1st day	33,814	24.7	34,088	24.9	34,499	25.2
14th day	25,053	18.3	27,928	20.4	22,999	16.8
28th day	11,363	8.3	23,615	17.25	13,553	9.9

Source: created by the author

By day 14, a reduction in wound area was observed across all wounds. The measured areas were as follows: wound No. 1 – 18.3 cm², wound No. 2 – 20.4 cm², and wound No. 3 (control) – 16.6 cm². These results indicate that the incisions made at the wound edges did not halt the contraction process but rather reduced its rate. Specifically, wound No. 1 showed a 25.9% reduction in area, wound No. 2 a reduction of 18.1%, and the control wound No. 3 a reduction of 33.3%. Between days 14 and 28, the contraction of wound edges continued, albeit at differing rates. Although more incisions were made on the edges of wound No. 1 than wound No. 2, its area continued to decrease, reaching 33.6% of its original size by day 28. This value was only slightly higher than that of the control wound (wound No. 3), which had reduced to 39.3% of its initial area. Notably, wound No. 2 preserved the largest residual area, maintaining 69.3% of its initial surface area by day 28. To assess the results, the relative change in wound area was evaluated as a percentage of the original wound size rather than using absolute values. These findings are presented in Table 2 and visualised in Figure 7.

Table 2. Wound area indicators in % relative to the initial value

	Wound No. 1	Wound No. 2	Wound No. 3
1st day	100	100	100
14th day	74.1	81.9	66.7
28th day	33.6	69.3	39.3

Source: created by the author

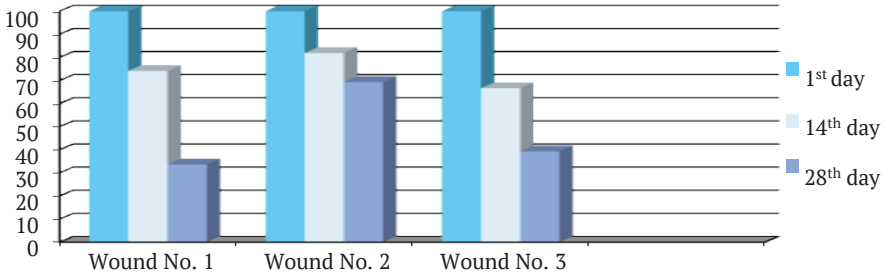

Figure 7. Wound area indicators in % relative to the initial value
Source: created by the author

The diagram presented in Figure 7 clearly illustrates the trend in wound surface area changes across the different wounds. A common pattern is evident: up to day 14, the rate of wound contraction is lower than during the period from day 14 to day 28. The findings demonstrate that disrupting the integrity of the wound edges can reduce the contraction process. However, analysis of the results for wound No. 1 indicates that a greater number of incisions, compared to wound No. 2, did not yield the expected outcome. The author suggests that it is not the number of incisions but rather their anatomical location that plays a more significant role in preventing or reducing the rate of wound edge contraction. Additionally, it is important to consider that the rate of contraction increases after day 14. Therefore, to effectively control this process, supplementary interventions at the wound edges may be required during this later stage of healing.

CONCLUSIONS

Wound contraction is a fundamental healing mechanism aimed at reducing the size of the tissue defect and, consequently, the volume of tissue requiring regeneration. In an experimental model involving the creation of a 5×5 cm excisional wound on the back of a pig, contraction occurs through the retraction of adjacent skin into the wound bed. This characteristic of full-thickness wound repair in porcine skin represents a major limitation of this model for studying wound regeneration processes relevant to humans. Despite the dense attachment of the dermis and subcutaneous fat to the superficial fascia in pigs, the skin still exhibits a significant capacity to migrate centrally during wound closure, even when all skin layers are involved. Reducing the contractile potential of the wound edges is therefore a key objective in developing a more representative experimental model of a full-thickness skin defect. One potential strategy is to disrupt the integrity of collagen fibres at the wound edge, which play a critical role in driving centripetal contraction of the wound. In this study, the application of tangential incisions at each corner of the wound – each up to 1 cm in length – proved effective in slowing the contraction process. By day 28, 69.3% of the original wound area was preserved in the intervention group, compared to only 39.3% in the control group. This proposed wound model allows for greater control over the contraction process and preserves a sufficient wound surface area for an adequate duration, thereby enabling meaningful evaluation of granulation tissue formation, re-epithelialisation, and scar development in response to the test treatment. During the healing process, the excisional full-thickness wound tends to assume an irregular, star-shaped configuration. This indicates that the surrounding skin does not contract uniformly; rather, skin edges equidistant from the wound centre move inward at varying speeds. This irregularity complicates planimetric analysis during experimental observation. It is also noteworthy that the inhibitory effect of edge disruption begins to diminish once the wound edges become closely opposed to the wound bed – a process that typically begins around days 10 to 12. From this point, contraction accelerates again, despite prior mechanical disruption of the wound margins. This observation highlights the need for further investigation into additional or sustained interventions that may be required beyond this critical period to effectively control wound narrowing and ensure the maintenance of a consistent wound area for experimental purposes.

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CONFLICT OF INTEREST

None.

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Особливості експериментального моделювання повношарової рани шкіри свині

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Анотація. В сучасному світі існує необхідність створення ревалентної експериментальної моделі рани шкіри, яка б максимально відтворювала процеси регенерації, що відбуваються в рані людини. Мета статті полягала у спробі створення попередження завчасної контракції країв рани при експериментальному моделюванні повношарової рани шкіри свині, що дозволить створити оптимальні умови для вивчення ефективності способу місцевого лікування. Проведено експериментальне дослідження на свині білої породи, вагою 15 кг. Повношарову рану розміром 5×5 см змодельовано на спині тварини під тіопентал-натрієвим наркозом з розрахунку 80 мг/кг. За допомогою панч-біопсії під загальним обезболенням вилучено тканини з рани, що фіксувалися в 10 % розчині нейтрального формаліну і ущільнювалися парафіном за стандартною методикою. Депарафінізовані зрізи було пофарбовано гематоксиліном і еозином. Для обчислення площі рани методом Монте-Карло було розроблено комп'ютерну програму мовою програмування Python. Використано графічний метод у вигляді діаграм для візуалізації результатів дослідження і спостереження тенденцій. Отримано можливість коригуючого впливу на процес контракції країв повношарового дефекту шкіри за допомогою надрізів. Найбільш оптимальним є варіант рани, що передбачає тангенціальні надрізи в кожному куті рани довжиною до 1 см. Застосована методика впливу на краї рани довзолила зменшити ступінь скорочення країв рани. На 28 добу спостереження площа рани становила 69,3 % від початкової, відповідно площа контрольної рани становила 39,3 % від її початкового значення. Для визначення ефективності лікування повношарової рани шкіри в експерименті на свині необхідно зберігати рану відповідної площі протягом не менше 28 діб для вивчення процесу формування рубцевої тканини. Запропонована модель рани дає можливість контролювати перебіг контракції, зберігати на необхідний час достатню площу ранової поверхні для вивчення процесу формування рубцевої тканини

Ключові слова: ексцизійна модель рани; модель на свині; контракція країв рани; площа рани; заживлення рани; дермальний матрикс