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Histological Assessment of the Effectiveness of Photobiomodulation Therapy in Healing Experimental Complicated Wounds

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Abstract: This work studied the effect of photobiomodulation (PBM) therapy on the healing of experimental complicated wounds, using histological examination as the gold standard to assess the treatment's usefulness and determine the effectiveness of selected parameters to minimize side effects. This study simulated chronic wounds in 48 rats. The animal's wounds in the experimental group were exposed to PBM. The semi-quantitative method evaluated the histological processes and structures, including the re-epithelialization stage, polymorphonuclear leukocytes (PMNLs), fibroblasts, new vessels, and new collagen. In the early stages of wound healing, the animals in the experimental group had less inflammation. The PMNL count was 1.50 times lower after 3 days and 1.70 times lower after 7 days compared to the control group. After 3 days, the number of fibroblasts was 1.83 times higher, the number of newly formed vessels was 1.24 times higher, and the number of collagen fibers was 1.75 times higher than in the control group. After 7 days, collagen levels were 1.29 times higher than in the control group. The timing of re-epithelialization and healing rates at 14 and 28 days were not significantly different between groups. Using PBM with these parameters can reduce inflammatory reactions, accelerate granulation tissue growth, and improve healing rates in the early stages of wound healing.

Keywords: photobiomodulation therapy; complicated wounds; histological assessment; rats.

1. Introduction

It is known that wound healing is a complex process involving many biological pathways and mechanisms. It is classified into different phases, including the hemostasis/inflammation stage, proliferation, and remodeling [1]. Healing of skin tissue is delayed when wounds do not go through this organized process, which can lead to chronic wounds [2]. During the formation of a chronic wound, disruptions in the relationship between endocrine and immune factors, as well as changes in metabolism, occur [3,4]. At the same time, in the case of extensive trauma, disruptions in regulatory mechanisms at the cellular level play a key role [5].

Due to the high prevalence of chronic wounds [6], the need for a multidisciplinary approach to assessing wound condition [7], the economic burden of treatment costs [8], etc., the development and improvement of innovative methods for healing skin wounds are relevant to medicine. For the treatment of wounds, for example, nanotherapy [9], extracts of xenogeneic origin [10], and physical methods of influencing the wound process [11] are used. Thus, the use of photobiomodulation (PBM) therapy is a promising strategy for both wound healing [12] and the treatment of many diseases, including neuroinfections [13], as well as for reducing acute and chronic pain [14].

A widely recognized mechanism underlying PBM is the effect of photons on cytochrome c oxidase [15]. Hypotheses related to the effects of light on ion channels and the cytoskeleton, biophoton production, and the properties of light and biological molecules exist [16]. The effects associated with PBM are considered at the molecular, cellular, and systemic levels [17].

Animal models are often used to study the complex mechanisms underlying wound healing. Rodent models allow the reproduction of key aspects of wound healing in humans and provide a platform to investigate various aspects of tissue repair, including cellular responses, molecular signaling, and dynamics of extracellular matrix remodeling. However, it should be considered that rodent wounds heal mainly by contraction, whereas in humans, re-epithelialization prevails. The wound-healing process in rodents is faster than in humans [18].

The use of rats in wound-healing models is more successful because larger wounds can be reproduced.

The first works on applying low-intensity laser radiation in wound healing were presented by Endre Mester. He published an article on the effects of low-level lasers on improving non-healing or slow-healing wounds [19]. Since then, large papers have been published on the effect of PBM on wound defects with different radiation parameters applied to both humans and animals, as well as in vitro. However, the mechanisms underlying PBM remain to be elucidated.

This work's purpose was to study the effect of PBM therapy on the healing of experimental complicated wounds using histological examination (a reliable method, as the results can be recorded photographically for evaluation by different experts).

2. Materials and Methods

2.1. Experimental design.

48 white rats at the age of 10 months, weighing 200-220 g, were used in the experimental studies. After a one-week adaptation period, the animals were randomized into two groups: control (Con) and experimental (Exp) [20]. Complicated wounds were simulated for rats of both groups after depilation, reproducing the conditions of impaired microcirculation and hypoxia. Wounds were induced into a 2 cm diameter circle in the proximal dorsum of the rat. Then, a perpendicular loop-shaped fasciocutaneous suture was placed along the wound edges. The superficial fascia of the wound bottom was excised with perpendicular cuts, forming 5 x 5 mm cells. The resulting cells were sutured with U-shaped sutures [21]. Surgical procedures were performed using anesthesia – intramuscular injection of Zoletil (tiletamine hydrochloride and zolazepam hydrochloride) (Virbac, France) at a concentration of 10 mg/kg body weight.

The wounds of animals in the experimental group were exposed to PBM. For this purpose, a laser device Lika-therapist M (Ukraine) with a wavelength of 660 nm, a power of 10 mW, an energy density of 0.5 J/cm^2, and an exposure time of 157 s was used. The impact on the wound defect was carried out through continuous-wave operation once a day for five consecutive days, starting 24 hours after modeling the wound. A distant method was used to illuminate the entire area of the wound. The control group rats were subjected to the same manipulations as the experimental group, but with the laser off (sham irradiation). The wounds remained open throughout the experiment.

2.2. Histological evaluation.

The animals were euthanized on days 3, 7, 14, and 28 of the experiment. A section of the wound was cut out for histological examination, including all its sections (central, main, and edge). The material was embedded in paraffin and stained with hematoxylin-eosin and Van Gieson's picrofuchsin. The preparations were analyzed and photographed using a PrimoStar microscope (Zeiss, Germany) with a digital camera.

The semi-quantitative method evaluated re-epithelialization stage, polymorphonuclear leukocytes (PMNL), fibroblasts, new vessels, and new collagen, according to the scale: 0, 1, 2, 3, 4 (Table 1) [22].

Table 1. Evaluation of histological parameters of healing wounds by the semi-quantitative method.

Scale	Reepithelization stage	Polymorphonuclear leukocytes	Fibroblasts	New vessels	Collagen
0	thickening of cut edges	absent	absent	absent	absent-granulation tissue
1	migration of cells (<50%)	mild-surrounding tissue	mild-surrounding tissue	mild-surrounding tissue	minimal-granulation tissue
2	migration of cells (≥50%)	mild-granulation tissue	mild-granulation tissue	mild-granulation tissue	mild-granulation tissue
3	bridging the excision	moderate-granulation tissue	moderate-granulation tissue	moderate-granulation tissue	moderate-granulation tissue
4	keratinization	marked-granulation tissue	marked-granulation tissue	marked-granulation tissue	marked-granulation tissue

2.3. Planimetry.

The measurement of the wound area was carried out in the control and experimental groups using digital planimetry. The wound area was measured from photographs using ImageJ image analysis software (NIH, USA) [23]. The relative wound area (S) was calculated according to the formula:

$$S(\%) = \frac{\mathrm{St}}{\mathrm{So}}\, x\, 100 \qquad (1)$$

Where *So* is the wound area after it has been inflicted, *St* is the surface area of the wound at a given healing period.

2.4. Statistical analysis.

The statistical analysis was performed by the one-way ANOVA using the Statistica software 12.0 (StatSoft, USA). To determine differences between groups, the Kruskal–Wallis rank-sum test was used, with post hoc comparisons performed using the Mann–Whitney U-test. Results were considered statistically significant at $P < 0.05$. Data were expressed as the means ± standard error of the mean (SE). Histogram plotting was performed in GraphPad Prism 7 (GraphPad Software, USA).

3. Results and Discussion

Figure 1 presents the change in the relative area of the wound surface in the control and experimental groups.

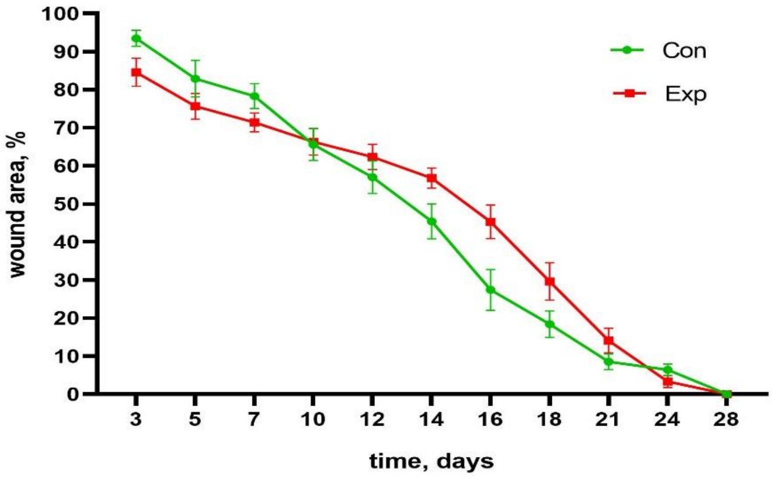

Figure 1. Change in the relative area of the wound surface in the control (Con) and experimental (Exp) groups of animals. The error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

3.1. Three days after surgery.

After 3 days, thickening of the epidermis along the wound edges was observed in most animals in both groups, due to cell proliferation in the basal epidermal layer. In some animals, there were signs of slight migration of keratinocytes from the wound edges to the center, without significant differences between groups.

The inflammatory reaction was significantly more pronounced in the control group of animals. PMNLs were observed in large quantities in the wound bed, as well as in areas of young granulation tissue in the form of diffuse and focal infiltration, forming a wide enough demarcation line between the scab and living tissues. In the experimental group of rats, the number of PMNL in all wound areas was significantly lower, 1.50 times ($p < 0.05$).

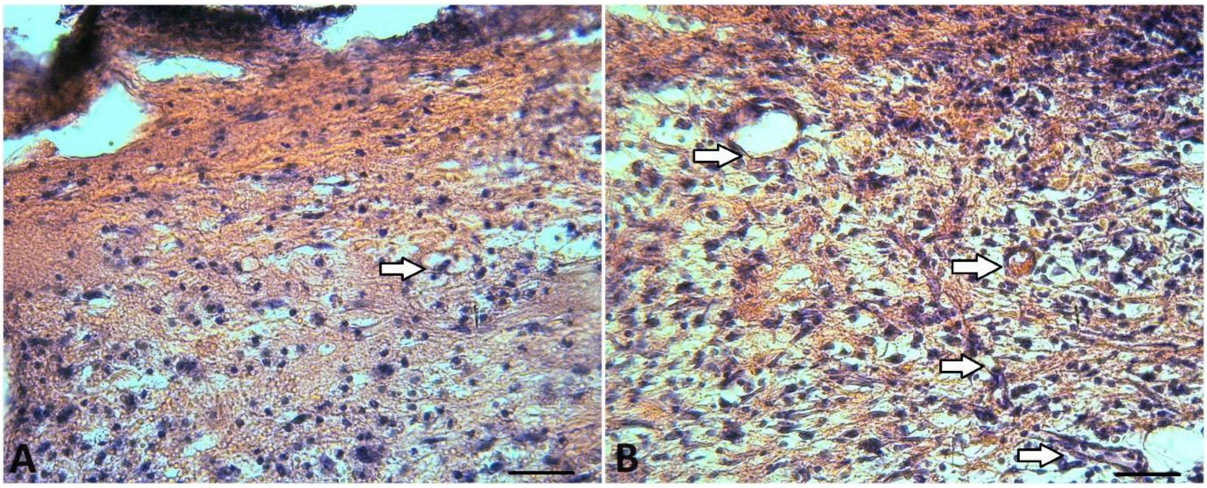

Figure 2. Wound area after 3 days in animals: (**a**) control group – against the background of fibrin threads, inflammatory elements, a few fibroblasts, and single capillaries (arrow); (**b**) experimental group – young granulation tissue with a large number of fibroblasts, capillaries (arrows), thin, chaotic collagen fibers. Hematoxylin and eosin; scale bar 50 µm.

The wound cavity in the control group was predominantly filled with fibrin fibers; isolated areas with signs of fibroblast proliferation and neoangiogenesis were noted. In animals of the experimental group, wound defects were filled with young granulation tissue. The semi-quantitative analysis also confirmed the great activity of reparative processes in rats receiving PBM therapy. In the experimental group, the number of fibroblasts was 1.83 times higher, the number of newly formed vessels was 1.24 times higher, and the number of collagen fibers was 1.75 times higher compared to the control group ($p < 0.05$) (Figures 2, 3).

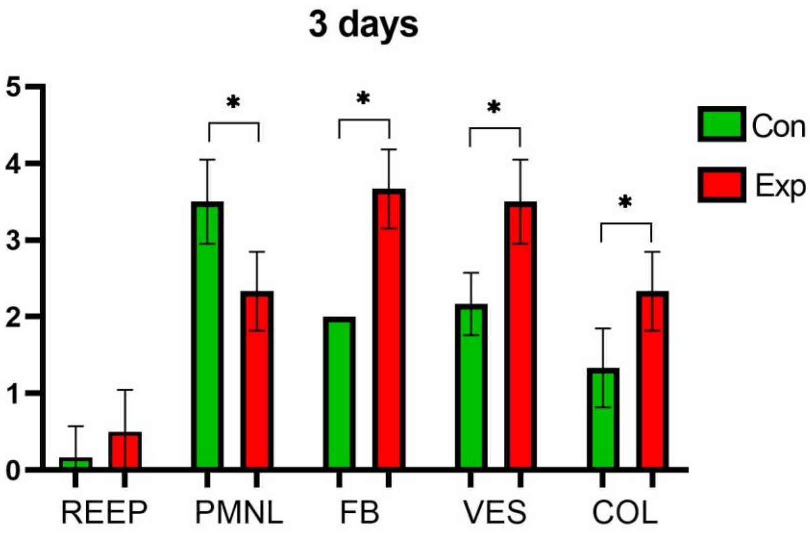

Figure 3. Results of semi-quantitative histological analysis of wounds in rats of the control (Con) and experimental (Exp) groups after 3 days: stage of re-epithelialization (REEP), number of polymorphonuclear leukocytes (PMNL), fibroblasts (FB), vessels (VES), collagen (COL); (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

3.2. Seven days after the surgery.

At this time, in wound samples from both groups of rats, signs of continued re-epithelialization and granulation tissue growth were noted. The organization of the extracellular matrix was more pronounced in the experimental group: the packing of collagen fibers was denser, their orientation parallel to the wound surface, and their number was greater, 1.29 times that of the control group ($p < 0.05$). The amount of PMNL, as in the previous period, was significantly higher in the control group, 1.7 times ($p < 0.05$) (Figures 4, 5).

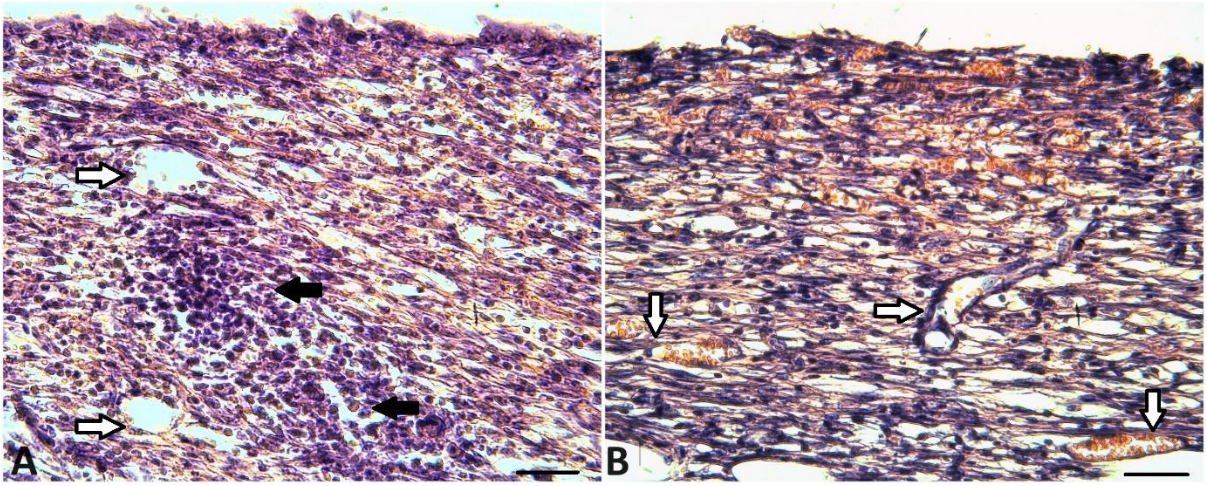

Figure 4. Wound areas after 7 days in animals: (**a**) control group – young granulation tissue with significant diffuse and focal (black arrows) inflammatory infiltration, fibroblasts, and capillaries (white arrows); (**b**) experimental group – granulation tissue with fibroblasts, capillaries (white arrows), and parallel bundles of collagen fibers. Hematoxylin and eosin; scale bar 50 μm.

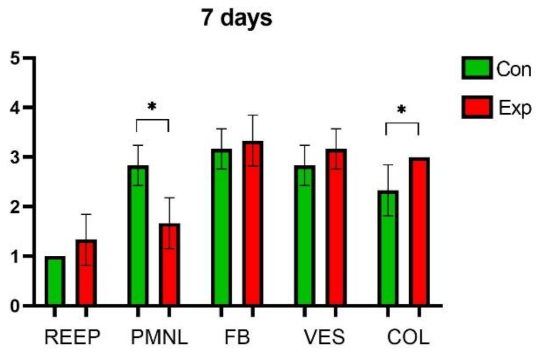

Figure 5. Results of semi-quantitative histological analysis of wounds in rats of the control (Con) and experimental (Exp) groups after 7 days: stage of re-epithelialization (REEP), number of polymorphonuclear leukocytes (PMNL), fibroblasts (FB), vessels (VES), collagen (COL); (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

3.3. Fourteen days after the surgery.

After 14 days, wound closure continued in both groups. In most animals, under a small scab, the central area of the wound remained uncovered. Wound samples from all animals showed signs of a remodeling phase during healing. Specifically, a decrease in the number of PMNs, fibroblasts, and vessels, as well as an increase in collagen in granulation tissue, with no significant differences between groups (Figures 6, 7).

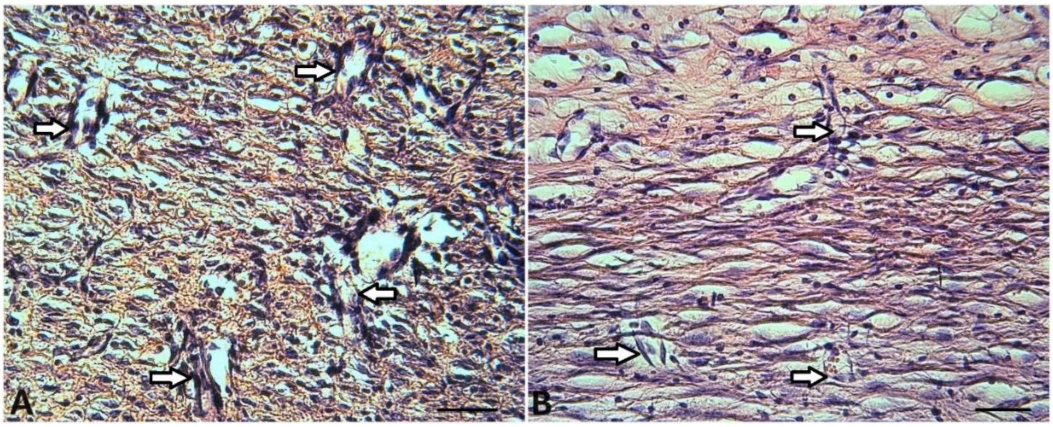

Figure 6. Wound areas after 14 days in animals of (**a**) the control; (**b**) experimental groups – maturing granulation tissue with bundles of collagen fibers and newly formed vessels (white arrows). Hematoxylin and eosin; scale bar 50 µm.

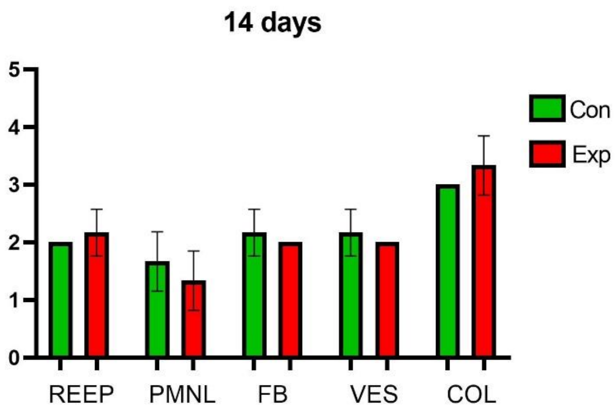

Figure 7. Results of semi-quantitative histological analysis of wounds in rats of the control (Con) and experimental (Exp) groups after 14 days: stage of re-epithelialization (REEP), number of polymorphonuclear leukocytes (PMNL), fibroblasts (FB), vessels (VES), collagen (COL); (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

3.4. Twenty-eight days after the surgery.

At the end of the experiment, all animals' wounds were completely epithelialized. Analysis of histological examination parameters revealed no significant differences between the control and experimental groups. In the wound samples of all animals, signs of completion of reparative processes in the marginal sections of the wounds and continuation of the remodeling phase in the central sections were observed. Here, newly formed vessels, fibroblasts, and inflammatory elements were preserved in small quantities (Figures 8, 9).

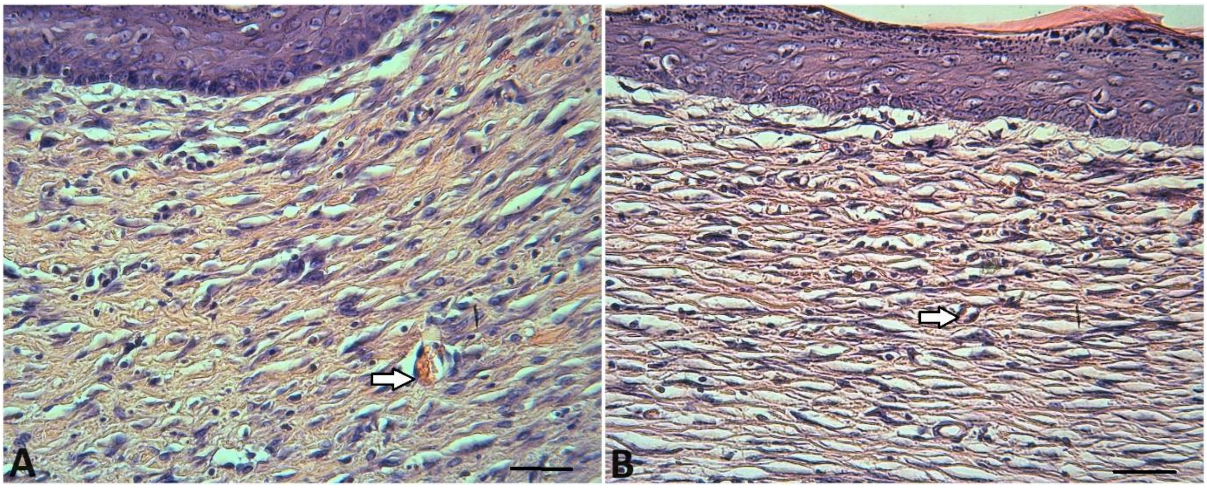

Figure 8. Areas of the central part of the wounds after 28 days in animals of **(a)** the control; **(b)** experimental groups - maturing granulation tissue with bundles of collagen fibers and single newly formed vessels (white arrows). Hematoxylin and eosin; scale bar 50 µm.

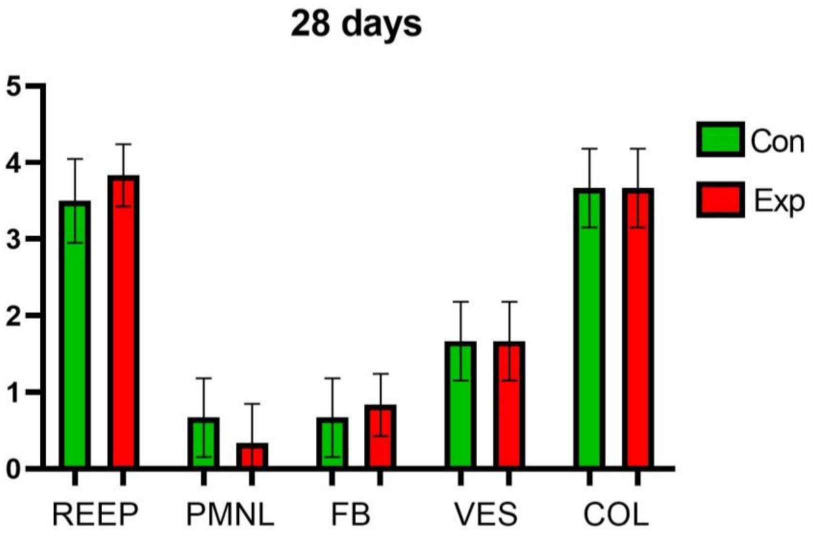

Figure 9. Results of semi-quantitative histological analysis of wounds in rats of the control (Con) and experimental (Exp) groups after 28 days: stage of re-epithelialization (REEP), number of polymorphonuclear leukocytes (PMNL), fibroblasts (FB), vessels (VES), collagen (COL); (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator ($n = 6$).

PBM therapy accelerates tissue regeneration. However, important parameters influencing treatment outcomes include the frequency of PBM therapy, the dose, and the method of energy delivery [24]. Therefore, an understanding of the applicability of the biphasic dose-response curve (Arndt-Schultz curve) to PBM parameters is necessary. There is an optimal "dose" value, most often determined by energy density (J/cm^2) [25]. In our work, the parameters of PBM therapy were selected based on the positive results of studies presented in the literature [26–29]. We also considered that the optimal therapeutic dose for laser therapy in an open wound is 0.5-1 J/cm^2 [30]. In our previous work, to improve wound healing, we used a wavelength of 660 nm, an energy density of 1 J/cm^2, and powers of 10 mW and 50 mW [31]. In the current study, an energy density of 0.5 J/cm^2 at a power of 10 mW was chosen to optimize the healing process.

The positive effect of PBM therapy in our study was noted mainly at the initial stages of wound healing, after 3 and 7 days. In the animals of the experimental group, the inflammatory reaction was less pronounced. This probably indicates earlier clearing of wound fluid containing tissue detritus and lytic enzymes of dead neutrophils. In turn, this promotes faster restoration of the structural integrity of tissues. Early fibroblast proliferation ensures collagen deposition, which forms the basis of the extracellular matrix. Signs of active capillary growth in the early stages contribute to trophic and oxygenation restoration of the injured area, which is especially important for chronic wounds. The positive effects of PBM have also been demonstrated in the literature. Thus, using laser irradiation 5 times every other day (compared to a single application) reduced inflammation and improved granulation tissue maturation and collagen deposition in a mouse wound-healing model. At the same time, an improvement in angiogenesis was shown on the 5th day of the experiment [32]. Other authors have shown that laser irradiation (635 nm at energy densities of 1 J/cm^2 and 3 J/cm^2) accelerates wound healing, especially in the early stages [33]. It was also demonstrated that the amount of collagen was high in the irradiation group on day 8 of PBM therapy for wound healing [34]. These data are similar to the results obtained in our work.

However, the PBM therapy regimen we studied appears to have a short-term effect, as positive differences at later stages of the reparative process were not observed compared with the control group. According to the literature, the increase in total collagen after irradiation with a 2 J/cm2 laser at 632 nm was statistically significant at 10 and 15 days after wounding compared with non-irradiated wounds [35]. Previously, we reported improved collagen structure in a group of animals after PBM therapy at the remodeling stage [36]. Another study showed that the number of blood vessels at day 28 was significantly increased in wounds treated with red light [37]. This is contrary to the data of the present study. The studies presented used different parameters of PBM therapy, making it difficult to compare the results. It should also be noted that experimental models studying the effects of PBM on chronic wound healing are presented mainly on diabetic rats. Most of the works contain information on using PBM in acute wounds.

Assessing the rate of wound closure, it should be noted that wound healing was significant until the 7th day of the experiment; further, no differences in wound contraction area were observed after PBM therapy. Similar results were obtained in a study in which, after using a laser (635 nm) to heal diabetic wounds in rats, a high percentage of wound closure after injury was obtained on the 6th day [38]. Our previous study showed decreased wound-healing time with PBM therapy (660 nm) up to day 7 of the experiment [39]. In a survey by Barbosa et al., when using PBM (658 nm) for wound healing, no difference in wound area was found on day 14 of the experiment [40]. Given the variety of experimental models and therapeutic parameters, the need to develop effective wound treatment protocols is urgent. Randomized trials are needed to optimize wound treatment and patient safety [41].

4. Conclusions

Our study demonstrated that using PBM therapy with these parameters can reduce inflammatory reactions and enhance granulation tissue growth and healing rates in the early stages of wound healing. Further research is needed to optimize dosimetry when using PBM therapy.

Difficulties in comparing PBM-treated studies stem from differences in PBM parameters, treatment protocols, and the biological target tissues. Despite the valuable results obtained experimentally, differences between animal and human species in physiologic and/or pathophysiologic aspects should be considered when planning clinical trials.

Author Contributions

Conceptualization, O.L. and S.P.; methodology, N.B.; validation, M.K.; formal analysis, M.K.; investigation, O.L. and I.T.; data curation, S.P.; writing—original draft preparation, N.B., M.K., and O.L.; writing—review and editing, O.L. and I.T.; visualization, O.L. and M.K.; supervision, S.P.; project administration, I.T. All the authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted following the “European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” (Strasbourg, 1986) and approved by the Commission on Ethics and Bioethics of Kharkiv National Medical University (protocol No. 17 dated 6 March 2024).

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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This research has no acknowledgment.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Fernández-Guarino, M.; Bacci, S.; Pérez González, L.A.; Bermejo-Martínez, M.; Cecilia-Matilla, A.; Hernández-Bule, M.L. The Role of Physical Therapies in Wound Healing and Assisted Scarring. *Int. J. Mol. Sci.* **2023**, *24*, 7487, https://doi.org/10.3390/ijms24087487.
2. Raziyeva, K.; Kim, Y.; Zharkinbekov, Z.; Kassymbek, K.; Jimi, S.; Saparov, A. Immunology of Acute and Chronic Wound Healing. *Biomolecules* **2021**, *11*, 700, https://doi.org/10.3390/biom11050700.
3. Pavlov, S.B.; Tamm, T.I.; Komisova, T.Y.; Babenko, N.M.; Kumechko, M.V.; Litvinova, O.B. The nature of changes in endocrine and immune factors at the initial stage of the formation of chronic wounds. *Modern Med. Technol.* **2023**, *2*, 34–39, https://doi.org/10.34287/MMT.2(57).2023.6.
4. Pavlov, S.; Babenko, N.; Kumetchko, M.; Litvinova, O.; Mikhaylusov, R. Features of metabolism in chronic wound remodelling. *Scr. Med.* **2024**, *55*, 53-61, https://doi.org/10.5937/scriptamed55-48179.
5. Babenko, N.M.; Litvinova, O.B.; Pavlov, S.B.; Kumechko, M.V.; Komarchuk, V.V. Problems of healing chronic wounds. *Modern Med. Technol.* **2023**, *3*, 66-70, https://doi.org/10.34287/MMT.3(58).2023.10.
6. Martinengo, L.; Olsson, M.; Bajpai, R.; Soljak, M.; Upton, Z.; Schmidtchen, A.; Car, J.; Järbrink, K. Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies. *Ann. Epidemiol.* **2019**, *29*, 8-15, https://doi.org/10.1016/j.annepidem.2018.10.005.
7. Lurin, I.A.; Khomenko, I.P.; Nehoduiko, V.V.; Tertyshnyi, S.V.; Makarov, V.V.; Kvasnevskyi, I.Y.; Kvasnevskyi, O.A. PROSPECTS OF THE HIGH-SPEED MULTIMEDIA DATA TRANSMISSION TECHNOLOGIES USE IN THE STRUCTURE OF THE SYSTEM FOR PROVIDING AID TO INJURED PERSONS HAVING A GUNSHOT DEFECT OF SOFT TISSUES. *Wiad. Lek.* **2023**, *76*, 1191-1198.
8. Olsson, M.; Järbrink, K.; Divakar, U.; Bajpai, R.; Upton, Z.; Schmidtchen, A.; Car, J. The humanistic and economic burden of chronic wounds: A systematic review. *Wound Repair Regen.* **2019**, *27*, 114-125, https://doi.org/10.1111/wrr.12683.
9. Prokopiuk, V.; Yefimova, S.; Onishchenko, A.; Kapustnik, V.; Myasoedov, V.; Maksimchuk, P.; Butov, D.; Bespalova, I.; Tkachenko, A. Assessing the Cytotoxicity of TiO_{2-x} Nanoparticles with a Different $Ti^{3+}(Ti^{2+})/Ti^{4+}$ Ratio. *Biol. Trace Elem. Res.* **2023**, *201*, 3117–3130, https://doi.org/10.1007/s12011-022-03403-3.
10. Vlasov, O.O.; Kovalov, G.O.; Myroshnychenko, M.S. MORPHOLOGICAL FEATURES OF A COLD SKIN WOUND UNDER THE INFLUENCE OF AN EXTRACT OF CRYOPRESERVED SKIN FRAGMENTS OF PIGLETS (EXPERIMENTAL STUDY). *Wiad. Lek.* **2020**, *73*, 306–311, https://doi.org/10.36740/WLek202002119.
11. Myronov, P.; Duzhyi, I.; Bugaiov, V.; Holubnycha, V.; Sikora, V.; Opanasyuk, A.; Romaniuk, A.; Pererva, O.; Pogorielov, M. Low-Frequency Ultrasound Reinforces Silver Nanoparticles Effect in Experimental Chronic Non-healing Purulent Wounds Treatment. *BioNanoScience* **2023**, *13*, 2337–2347, https://doi.org/10.1007/s12668-023-01195-x.
12. Baracho, V.d.S.; Chaves, M.E.d.A.; Huebner, R.; Oliveira, M.X.; Ferreira, P.H.d.C.; Lucas, T.C. Phototherapy (cluster multi-diode 630 nm and 940 nm) on the healing of pressure injury: A pilot study. *J. Vasc. Nurs.* **2021**, *39*, 67-75, https://doi.org/10.1016/j.jvn.2021.06.002.
13. Cardoso, F.d.S.; Salehpour, F.; Coimbra, N.C.; Gonzalez-Lima, F.; Gomes da Silva, S. Photobiomodulation for the treatment of neuroinflammation: A systematic review of controlled laboratory animal studies. *Front. Neurosci.* **2022**, *16*, 1006031, https://doi.org/10.3389/fnins.2022.1006031.
14. Wu, D.; Zhao, Y.L.; Dai, R.J.; Rong, P.J.; Wang, Y. Application of Photobiomodulation Therapy in Acupuncture. *World J. Tradit. Chin. Med.* **2022**, 8, 491–496, https://doi.org/10.4103/wjtcm.wjtcm_12_22.
15. Freitas, L.F.d.; Hamblin, M.R. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J. Sel. Top. Quantum Electron.* **2016**, *22*, 348-364, https://doi.org/10.1109/JSTQE.2016.2561201.
16. Liebert, A.; Capon, W.; Pang, V.; Vila, D.; Bicknell, B.; McLachlan, C.; Kiat, H. Photophysical Mechanisms of Photobiomodulation Therapy as Precision Medicine. *Biomedicines* **2023**, *11*, 237, https://doi.org/10.3390/biomedicines11020237.
17. da Silva, T.G.; Ribeiro, R.S.; Mencalha, A.L.; de Souza Fonseca, A. Photobiomodulation at molecular, cellular, and systemic levels. *Lasers Med. Sci.* **2023**, *38*, 136, https://doi.org/10.1007/s10103-023-03801-6.
18. Choudhary, V.; Choudhary, M.; Bollag, W.B. Exploring Skin Wound Healing Models and the Impact of Natural Lipids on the Healing Process. *Int. J. Mol. Sci.* **2024**, *25*, 3790, https://doi.org/10.3390/ijms25073790.

19. Mester, E.; Mester, A.F.; Mester, A. The biomedical effects of laser application. *Lasers Surg. Med.* **1985**, *5*, 31–39, https://doi.org/10.1002/lsm.1900050105.
20. Singh, K.; Yadav, V.B.; Yadav, U.; Nath, G.; Srivastava, A.; Zamboni, P.; Kerkar, P.; Saxena, P.S.; Singh, A.V. Evaluation of biogenic nanosilver-acticoat for wound healing: A tri-modal in silico, in vitro and in vivo study. *Colloids Surf. A: Physicochem. Eng. Asp.* **2023**, *670*, 131575, https://doi.org/10.1016/j.colsurfa.2023.131575.
21. Pavlov, S.B.; Babenko, N.M.; Kumetchko, M.V.; Litvinova, O.B.; Mikhaylusov, R.N. Experimental Study of the Effect of Photobiomodulation Therapy on the Regulation of the Healing Process of Chronic Wounds. *Int. J. Photoenergy* **2021**, *2021*, 3947895, https://doi.org/10.1155/2021/3947895.
22. Gal, P.; Kilik, R.; Mokry, M.; Vidinsky, B.; Vasilenko, T.; Mozes, S.; Bobrov, N.; Tomori, Z.; Bober, J.; Lenhardt, L. Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semi-quantitative and quantitative histological assessments. *Vet Med* **2008**, *53*, 652-659, https://doi.org/10.17221/1973-VETMED.
23. Singh, A.V.; Bansod, G.; Mahajan, M.; Dietrich, P.; Singh, S.P.; Rav, K.; Thissen, A.; Bharde, A.M.; Rothenstein, D.; Kulkarni, S.; Bill, J. Digital Transformation in Toxicology: Improving Communication and Efficiency in Risk Assessment. *ACS Omega* **2023**, *8*, 21377-21390, https://doi.org/10.1021/acsomega.3c00596.
24. de Castro, J.R.; da Silva Pereira, F.; Chen, L.; Arana-Chavez, V.E.; Ballester, R.Y.; DiPietro, L.A.; Simões, A. Improvement of full-thickness rat skin wounds by photobiomodulation therapy (PBMT): A dosimetric study. *J. Photochem. Photobiol. B: Biol.* **2020**, *206*, 111850, https://doi.org/10.1016/j.jphotobiol.2020.111850.
25. Huang, Y.-Y.; Sharma, S.K.; Carroll, J.; Hamblin, M.R. Biphasic Dose Response in Low Level Light Therapy – an Update. *Dose Response* **2011**, *9*, https://doi.org/10.2203/dose-response.11-009.Hamblin.
26. Chen, Q.; Yang, J.; Yin, H.; Li, Y.; Qiu, H.; Gu, Y.; Yang, H.; Xiaoxi, D.; Xiafei, S.; Che, B.; Li, H. Optimization of photo-biomodulation therapy for wound healing of diabetic foot ulcers *in vitro* and *in vivo*. *Biomed. Opt. Express* **2022**, *13*, 2450-2466, https://doi.org/10.1364/BOE.451135.
27. Solmaz, H.; Ulgen, Y.; Gulsoy, M. Photobiomodulation of wound healing via visible and infrared laser irradiation. *Lasers Med. Sci.* **2017**, *32*, 903–910, https://doi.org/10.1007/s10103-017-2191-0.
28. Esmaeelinejad, M.; Bayat, M. Effect of low-level laser therapy on the release of interleukin-6 and basic fibroblast growth factor from cultured human skin fibroblasts in normal and high glucose mediums. *J. Cosmet. Laser Ther.* **2013**, *15*, 310–317, https://doi.org/10.3109/14764172.2013.803366.
29. Lopes-Martins, R.Á.B.; Marcos, R.L.; Leonardo, P.S.; Prianti, A.C.; Muscará, M.N.; Aimbire, F.; Frigo, L.; Iversen, V.V.; Bjordal, J.M. Effect of low-level laser (Ga-Al-As 655 nm) on skeletal muscle fatigue induced by electrical stimulation in rats. *J. Appl. Physiol.* **2006**, *101*, 283-288, https://doi.org/10.1152/japplphysiol.01318.2005.
30. Tunér, J.; Hode, L. Laser Therapy — Clinical Practice and Scientific Background. Prima Books: Grängesberg, Sweden, **2002**.
31. Sergey, P.; Nataliia, B.; Marina, K.; Olga, L.; Viktor, K. EFFECT OF PHOTOBIOMODULATION THERAPY ON THE PROCESSES OF REPAIR OF COMPLEX WOUNDS IN AN EXPERIMENTAL STUDY. *Zdravotnicke listy* **2023**, *11*, 56–62.
32. Gómez García, F.J.; Del Vecchio, A.; Romeo, U.; Martínez Díaz, F.; García Carrillo, N.; Camacho Alonso, F. Study of the Effect of Photobiomodulation on a Skin Repair Model in SKH-1 Mice. *Photobiomodulation Photomed. Laser Surg.* **2022**, *40*, 325-333, https://doi.org/10.1089/photob.2021.0158.
33. Solmaz, H.; Dervisoglu, S.; Gulsoy, M.; Ulgen, Y. Laser biostimulation of wound healing: bioimpedance measurements support histology. *Lasers Med. Sci.* **2016**, *31*, 1547–1554, https://doi.org/10.1007/s10103-016-2013-9.
34. Wang, Z.-X.; Kim, S.-H. Effect of Photobiomodulation Therapy (660 nm) on Wound Healing of Rat Skin Infected by *Staphylococcus*. *Photobiomodul. Photomed. Laser Surg.* **2020**, *38*, 419–424, https://doi.org/10.1089/photob.2019.4754.
35. Prabhu, V.; Rao, B.S.S.; Rao, A.C.K.; Prasad, K.; Mahato, K.K. Photobiomodulation invigorating collagen deposition, proliferating cell nuclear antigen and Ki67 expression during dermal wound repair in mice. *Lasers Med. Sci.* **2022**, *37*, 171-180, https://doi.org/10.1007/s10103-020-03202-z.
36. Pavlov, S.; Babenko, N.; Kumetchko, M.; Litvinova, O.; Valilshchykov, M. Features of cellular and molecular mechanisms of re-gulation of reparative processes in chronic wounds using photobiomodulation therapy. *Folia Med.* **2022**, *64*, 260–266, https://doi.org/10.3897/folmed.64.e61539.

37. Dungel, P.; Sutalo, S.; Slezak, C.; Keibl, C.; Schädl, B.; Schnidar, H.; Metzger, M.; Meixner, B.; Hartmann, J.; Oesterreicher, J.; Redl, H.; Slezak, P. Wavelength-Dependent Effects of Photobiomodulation for Wound Care in Diabetic Wounds. *Int. J. Mol. Sci.* **2023**, *24*, 5895, https://doi.org/10.3390/ijms24065895.
38. Wu, X.; Alberico, S.; Saidu, E.; Rahman Khan, S.; Zheng, S.; Romero, R.; Sik Chae, H.; Li, S.; Mochizuki, A.; Anders, J. Organic light emitting diode improves diabetic cutaneous wound healing in rats. *Wound Repair Regen.* **2015**, *23*, 104-114, https://doi.org/10.1111/wrr.12258.
39. Pavlov, S.B.; Babenko, N.M.; Kumetchko, M.V.; Litvinova, O.B.; Semko, N.G.; Mikhaylusov, R.N. THE INFLUENCE OF PHOTOBIOMODULATION THERAPY ON CHRONIC WOUND HEALING. *Rom. Rep. Phys.* **2020**, *72*, 609.
40. Barbosa, L.S.; Parisi, J.R.; Viana, L.d.C.; Carneiro, M.B.; Silva, J.R.T.d.; Silva, M.L.d.; Novaes, R.D.; Sousa, L.d. The photobiomodulation (658, 830 and 904nm) on wound healing in histomorphometric analysis. *Fisioter. Mov.* **2020**, *33*, e003318, https://doi.org/10.1590/1980-5918.033.AO18.
41. Singh, A.V.; Gemmati, D.; Kanase, A.; Pandey, I.; Misra, V.; Kishore, V.; Jahnke, T.; Bill, J. Nanobiomaterials for vascular biology and wound management: A review. *Veins Lymphat.* **2018**, *7*, 7196-7134, https://doi.org/10.4081/vl.2018.7196.

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