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Original Paper

ROLE OF BIOACTIVE MOLECULES IN CHRONIC WOUND HEALING PROCESSES USING PHOTOBIOMODULATION THERAPY

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Photobiomodulation (PBM) therapy is used to influence the reparative process in treating chronic wounds. The cellular and molecular mechanisms underlying PBM with tissue interaction are poorly understood. Understanding wound-healing processes with PBM therapy will enable optimisation of PBM parameters and the development of effective strategies for managing chronic wounds. This work aims to study the influence of bioactive molecules on the reparative processes of chronic wounds using PBM therapy. For the experimental study, 18 rats were randomised into intact, control, and experimental groups. Chronic wounds were induced in the control and experimental groups. PBM therapy was used in the experimental group. On the seventh day of the experiment, the animals were euthanised. The enzyme immunoassay method determined the concentration of interleukin-8, transforming growth factor-β1, platelet-derived growth factor, and amino-terminal propeptide of type III procollagen in blood serum. The present study showed that PBM therapy promotes wound healing by regulating bioactive molecules at the stage of inflammation conversion to the remodelling stage.

Keywords: photobiomodulation, reparative process, treating wounds, growth factors, interleukins.

INTRODUCTION

Wound healing is a physiological process with four sequential and overlapping stages: haemostasis, inflammation, proliferation, and remodelling. Healing and remodelling are critically dependent on controlling inflammation (Gao *et al.*, 2024). Wound healing and tissue regeneration are complex cellular processes involving the interaction of growth factors, cytokines, and other signalling molecules. By regulating the levels of cytokines and growth factors, it is possible to influence the wound-healing process (Kumechko *et al.*, 2025). Disruptions in the interactions between biomolecules at any of the stages of wound healing can lead to either chronic wound formation or fibrosis. Many factors contribute to the chronicisation of reparative processes: advanced age, comorbidities, endocrine disorders, obesity, etc. (Pavlov *et al.*, 2022; Lurin *et al.*, 2024).

Innovative treatment strategies are being utilised to influence the reparative process, such as nanotherapy, stem cell therapy, and 3D bioprinted skin (Kolimi *et al.*, 2022). Thus, photobiomodulation (PBM) therapy is used to heal tissue and reduce inflammation and pain (Babtan *et al.*, 2022; Pavlov *et al.*, 2024). PBM therapy is used to treat a wide range of diseases, including neurological diseases (Chamkouri *et al.*, 2024) and dental problems (Sena and Meira, 2024). PBM is also used for rehabilitation (Lawrence and Sorra, 2024), etc. PBM enhances the activity of mitochondrial cytochrome c oxidase and ATP production, promotes regeneration, regulates metabolism, and has an anti-inflammatory effect (Shi *et al.*, 2026).

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The cellular and molecular mechanisms underlying the PBM with tissue interaction are poorly understood. This leads to variability in the PBM parameters used. Understanding the processes of wound healing using PBM therapy will allow optimisation of the applied parameters of PBM therapy and the development of effective strategies for managing chronic wounds.

The aim of the work was to study the role of bioactive molecules interleukin-8 (IL-8), TGF-β1, platelet-derived growth factor (PDGF), and amino-terminal propeptide of type III procollagen (PIIINP) in influencing the development of reparative processes of chronic wounds using PBM therapy.

MATERIALS AND METHODS

The experiment involved 18 male rats from the WAG line weighing about 230 g and aged 8–9 months. The animals were housed in the Kharkiv National Medical University animal facility. During the acclimatisation period, six animals were housed in each cage; after surgery, one animal was housed per cage. The conditions for keeping laboratory animals included a natural light regime, an optimal temperature (20–22 °C), a standard diet, and free access to water (Guide for the Care and Use of Laboratory Animals, 2011).

The animals were randomised into intact, control, and experimental groups of six rats. Rats in the control and experimental groups were induced to develop chronic wounds with reproduction of hypoxic conditions and microcirculatory disturbance (Pavlov *et al.*, 2024). For anaesthesia, an intramuscular injection of zoletil (tiletamine hydrochloride and zolazepam hydrochloride) (Virbac, France) at a concentration of 10 mg/kg body weight was used. PBM therapy was used in the experimental group. The laser device Lika Therapist M (Ukraine) was used. The entire wound surface was irradiated with a wavelength of 660 nm, a power of 10 mW, and an energy density of 1 J/cm^2; the duration of a single session was 314 seconds. The laser tip was held perpendicular to the wound surface at a distance of 3.53 cm. Therapy was applied for five days and started 24 hours after wound induction. Thus, the total energy delivered to the wound was 15.7 J. Wound defects of animals in the control group received fictitious laser exposure. On the seventh day of the experiment, the rats were euthanised. Blood was withdrawn from the heart by the open method. The enzyme immunoassay method determined the concentration of IL-8, TGF-β1, PDGF, and PIIINP in blood serum using eBioscience kits (USA).

A section of the animal's wound, including the central, main, and marginal sections, was excised for histological examination. The material was embedded in paraffin and stained with haematoxylin-eosin and Van Gieson's picrofuchsin. The preparations were analysed and photographed using a PrimoStar microscope (Zeiss, Germany) and a digital camera. The semi-quantitative method evaluated histological processes and structures: re-epithelialisation stage, polymorphonuclear leucocytes (PMNL), fibroblasts, new vessels, and new collagen. The sections were assessed blindly, according to the scale: 0, 1, 2, 3, 4 (Gal *et al.*, 2008).

Statistical analysis

Statistical analysis was performed using Statistica 12.0 software (StatSoft Inc., USA). The normality of data distribution within groups (n = 6) was formally tested using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. For data demonstrating a normal distribution (such as biochemical parameters IL-8, TGF-β1, PDGF, and PIIINP), parametric methods were applied. Differences among groups were evaluated using one-way Analysis of Variance (ANOVA) followed by the Tukey's post-hoc test for pairwise comparisons. These data are presented as mean ± standard error of the mean (mean ± SE).

For data that did not meet the assumptions of normality or were measured on an ordinal scale (semi-quantitative histological scoring), non-parametric statistical methods were utilised. Intergroup comparisons were conducted using the Kruskal–Wallis test followed by the Dunn's post-hoc test, with values expressed as median and interquartile range [Me (Q1–Q3)].

Differences were considered statistically significant at $p < 0.05$. Graphical visualisation and histogram plotting were performed using GraphPad Prism 9.0 (GraphPad Software, USA).

RESULTS

Figure 1 presents the concentrations of bioactive molecules in the animals' blood serum on day 7 of the experiment. The concentration of TGF-β1 in the blood serum of control animals, compared to intact animals, was 1.52 times ($p < 0.01$) lower seven days after surgery (Fig. 1A). TGF-β1 concentration in the group of animals with PBM was 1.33-fold larger ($p < 0.01$) compared to intact animals. At the same time, on the seventh day of the experiment, the level of TGF-β1 in the experimental group was 2.02 times larger ($p < 0.001$) compared to the control group. The level of PDGF in the serum of animals in the control group, compared to intact animals, was lower ($p > 0.05$) at seven days after surgery (Fig. 1B). The concentration of PDGF in the serum of experimental animals with PBM was 1.52 times larger ($p < 0.05$) compared to intact animals. The PDGF lev-

el in the serum of experimental animals was 1.65-fold larger ($p < 0.05$) compared to control animals. It was found that the IL-8 level in the serum of animals of the control and experimental groups was not significantly lower ($p > 0.05$) compared to similar parameters of intact animals (Fig. 1C). PIIINP concentration was 1.73-fold higher ($p < 0.05$) and 2.28-fold higher ($p < 0.001$) compared to intact animals in the control and PBM animal groups, respectively (Fig. 1D), but there were no significant differences in the levels of this indicator in the control and experimental groups on the seventh day of the experiment.

Figure 2 presents the results of a semi-quantitative histological analysis of wounds in rats of the control and experimental groups after seven days of the experiment.

In seven days after chronic wound modelling in rats of the control and experimental groups, partial epithelisation of wound defects was observed. The wound cavities were filled with young granulation tissue with large amounts of fibroblasts and newly formed vessels. Signs of inflammation were significantly more pronounced in the control group: the quantity of PMNL here was 1.71 times higher than in the experimental group ($p < 0.05$). Wound samples from animals receiving PBM therapy were distinguished by a greater number of newly formed collagen — 1.29 times more than in the control group ($p < 0.05$) (Fig. 2). At the same time, the experimental group showed a denser packing of collagen fibre bundles and their ordered arrangement parallel to the wound surface (Fig. 3).

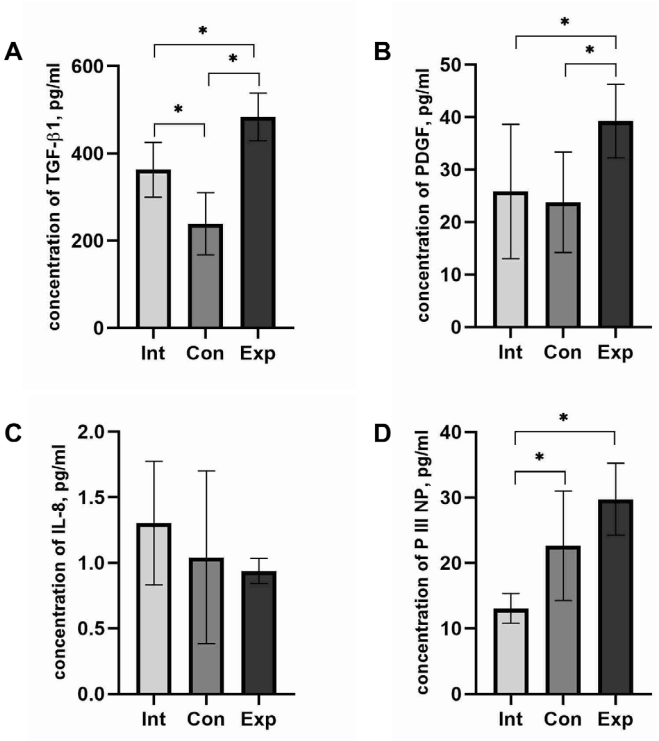


Fig. 1. Levels of the studied indicators in the blood serum of animals: (A) TGF-β1, (B) PDGF, (C) IL-8, (D) PIIINP; Int – intact group, Con – control group, Exp – experimental group; differences are significant relative to intact animals and the control group (*), ($p < 0.05$); the error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

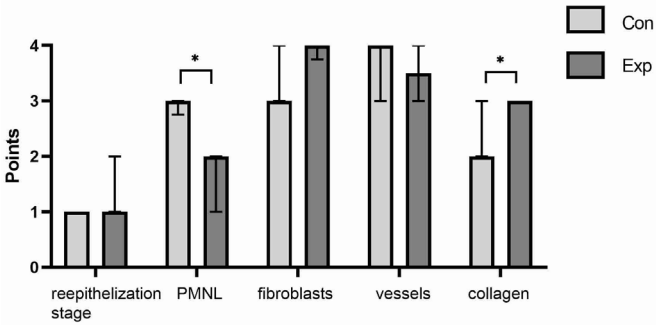


Fig. 2. Semi-quantitative histological analysis data on the 7th-day experiment; Con – control group, Exp – experimental group; differences are significant relative to the control group (*), ($p < 0.05$); bars and error bars represent median values and interquartile range for each indicator (n = 6).

DISCUSSION

It is known that the inflammatory phase is tightly controlled and at the cellular and molecular levels is associated with the proliferative phase of wound healing (El Ayadi *et al.*, 2020). In our work, wound healing was considered at the stage of transition from inflammation to the proliferation stage. Histological analysis performed in our study showed that using PBM therapy contributed to reducing inflammation at this stage. We have previously shown a reduced wound surface area

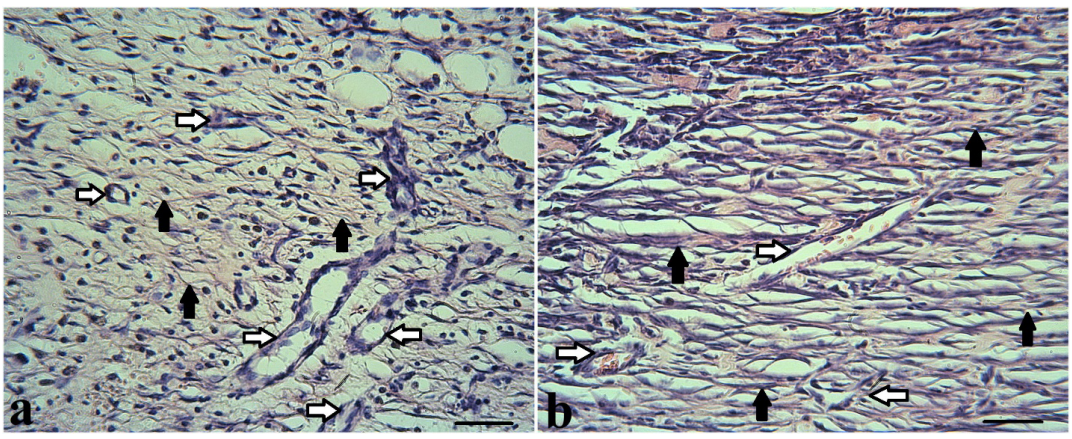


Fig. 3. Microphotographs of wound sites in rats, at high magnification, on the 7th day of the experiment: (a) control animal and (b) experimental animal: newly formed capillaries (white arrows), bundles of collagen fibres (black arrows). Stained with haematoxylin and eosin. Magnification 400×. Scale bar: 50 μm.

in the experimental group in which PBM therapy was used (Pavlov *et al.*, 2020).

The therapeutic efficacy of PBM at a wavelength of 660 nm is primarily mediated by the absorption of light photons by intracellular chromophores, notably cytochrome c oxidase in the mitochondrial respiratory chain. This activation triggers an increase in ATP production, modulation of reactive oxygen species (ROS), and activation of downstream signalling cascades, ultimately stimulating cellular proliferation and the secretion of bioactive mediators (Shi *et al.*, 2026).

PBM may accelerate wound healing by stimulating the production of certain mediators. Thus, TGF-β exerts a pleiotropic effect on wound healing by recruiting neutrophils to the wound site, stimulating the production of the extracellular matrix and angiogenesis, and modulating the immune response (Wong *et al.*, 2025). It is known that one of the mechanisms of PBM interaction with tissue is associated with selective activation of endogenous, latent TGF-β1 (Arany, 2022). PBM-activated TGF-β1 has also been shown to promote wound healing (Khan *et al.*, 2021). In our study, PBM therapy promoted an increase in TGF-β1 levels. This is consistent with the results of a paper in which TGF-β1 levels during burn wound healing in an animal model after laser treatment were higher compared to the false treatment group (Alves *et al.*, 2022).

In our study, PDGF levels were larger in the serum of animals in the experimental group under the influence of PBM therapy. PDGF is known to be an important growth factor that regulates inflammation, re-epithelialisation, collagen deposition, and tissue remodelling (Smith and Rai, 2024). Similar to our data, the literature shows an increase in PDGF-BB levels in patients with palate wounds treated with PBM (Keskiner *et al.*, 2016). Since TGF-β and PDGF promote fibroblast infiltration, thereby enhancing extracellular matrix production, the higher levels of these growth factors in the group of animals with PBM directly correlate with our histological findings, which showed accelerated re-epithelialisation, advanced fibroblast proliferation, and enhanced neovascularisation.

IL-8 levels after exposure to PBM therapy did not differ among treatments in our work. While TGF-β1 and PDGF are fibrogenic mediators, IL-8 is a proinflammatory cytokine that promotes angiogenesis and fibroblast migration (Matsui *et al.*, 2024). The literature demonstrated a significant decrease in IL-8 (CINC-1) concentration on day 7 after exposure to PBM in aged rats with wounds (Fiorio *et al.*, 2017). The absence of significant IL-8 modulation in our study on day 7 may indicate that the acute neutrophil-driven inflammatory phase had already resolved, as a result of which the healing process had largely transitioned to the stage of tissue repair and remodelling.

Exposure to PBM therapy showed a trend toward higher PIIINP levels in the experimental group, possibly reflecting tissue repair after surgery or collagen turnover. PIIINP appears in the bloodstream during collagen formation and degradation (Cowan and Moncrieffe, 2022). PIIINP arises from the extracellular conversion of type III procollagen to type III collagen (Biasucci *et al.*, 2021). Our histological analysis showed an enhanced collagen formation in the group of animals in which PBM therapy was used compared to the control group. An increase in the number of collagen fibres was demonstrated in our previous study when using PBM with a lower energy density for wound treatment (Litvinova *et al.*, 2025). Taken together, the elevation of systemic growth factors alongside histological evidence of newly formed vessels and structured matrix confirms that PBM (660 nm, 1 J/cm^2) effectively accelerates the shift from chronic tissue hypoxia to active tissue synthesis.

STUDY LIMITATIONS

Several limitations of the present study should be acknowledged. First, the sample size was relatively small (n = 6 animals per group), which was selected in accordance with the ethical 3Rs principles (Reduction) to minimise animal use while maintaining sufficient statistical power to detect primary differences. Second, tissue sampling and biomarker analyses were performed at a single time point (day 7 post-induction), which captures the transition phase from inflammation to proliferation but limits the evaluation of early acute responses or long-term scar remodelling. Third, while serum ELISA analysis of TGF-β1, PDGF, IL-8, and PIIINP alongside routine histology provided valuable insights into systemic mediator levels and matrix structure, local tissue protein expression was not evaluated using immunohistochemistry, and a broader panel of early inflammatory cytokines (such as IL-1β and IL-10) was not included. Future research utilising larger cohorts, multi-time-point sampling, immunohistochemical tissue mapping, and an expanded molecular panel is warranted to fully validate these findings and elucidate the detailed temporal dynamics of PBM therapy.

CONCLUSIONS

Our preliminary findings suggest that PBM therapy (660 nm, 10 mW, 1 J/cm^2) may accelerate chronic wound healing under hypoxic microcirculatory conditions by modulating bioactive molecules during the transition from the inflammatory phase to the proliferation and remodelling stages.

Specifically, PBM exposure was associated with elevated serum levels of TGF-β1 and PDGF, which play key roles in regulating cellular repair and tissue matrix synthesis. Histological analysis supported these biochemical observations, demonstrating reduced leucocyte infiltration, accelerated re-epithelialisation, advanced fibroblast proliferation, and enhanced structured collagen fibre deposition in the PBM-treated group.

Overall, these findings highlight the therapeutic potential of PBM as a promising non-invasive intervention for chronic wound management. However, further studies with larger cohorts and expanded follow-up periods are required to fully elucidate the precise systemic and molecular mechanisms involved.

ETHICS

The experiments were carried out in compliance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council, of 22 September 2010, on the Protection of Animals Used for Scientific Purposes (Directive 2010/63/EU, 2010), and the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV dated 02.21.2006), by the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (European Convention, 1986), as well as by the requirements of the Ethics and Bioethics Committee of Kharkiv National Medical University (Minutes No. 8, dated 17 October 2025).

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BIOAKTĪVO MOLEKULU LOMA HRONISKU BRŪČU DZĪŠANAS PROCESOS, IZMANTOJOT FOTOBIOMODULĀCIJAS TERAPIJU

Pētījuma mērķis bija izpētīt bioaktīvo molekulu ietekmi uz hronisku brūču atjaunošanās procesiem, izmantojot fotobiomodulācijas (PBM) terapiju. Eksperimentā ar žurkām parādīts, ka PBM terapija veicina brūču dzīšanu, regulējot bioaktīvās molekulas pārejas posmā no iekaisuma uz remodelācijas fāzi.