

Photobiomodulation therapy enhances pericyte coverage during skin regeneration in a murine diabetic model

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ABSTRACT

Photobiomodulation (PBMT) speeds up wound healing, partly by attracting pericytes. However, its specific mechanisms in a diabetic setting are still not well understood. We studied tissue regeneration after PBMT using a transgenic mouse model (NG2⁺DsRed/Nestin⁺GFP) with streptozotocin-induced Type 1 diabetes. PBMT was applied daily (660 nm, 20 mW, 7 s, 0.14 J, 0.71 W/cm², 5 J/cm²). Our results showed increased lumen area of pericyte-covered vessels and significant flow of perivascular and neural progenitor cells in PBMT-treated wounds. We also saw an increase in the pro-resolving cytokine IL-1RA after irradiation. PBMT did not change levels of GLUT1, TNF, IL-1 α , or NF- κ B in the chronic inflammatory environment. Diabetic cells treated with PBMT showed limited proliferation and migration but had improved ability for adipogenic differentiation. Despite only modest changes in the inflammatory microenvironment, photobiomodulation notably accelerates tissue repair by directly encouraging pericyte and neural progenitors entry into the wound bed.

1. Introduction

Diabetes mellitus (DM) is characterized by persistent hyperglycemia [1]. Although glycemic control can often be achieved with medication, improving delayed wound healing remains difficult due to the ongoing damage to the vascular endothelium [2]. Pericytes and endothelial cells are essential components of the endothelium, which serves as the interface between blood and tissue [3]. Pericytes, in particular, help with angiogenesis, maintaining vascular integrity, and regulating blood flow, thus playing a vital role in microvascular health. In diabetic

patients, pericyte coverage is decreased compared to healthy individuals, which may contribute to impaired wound healing [4,5].

Hyperglycaemia triggers multiple cellular processes, leading to an imbalance in free radical levels and activating the (Nuclear Factor - κ B) NF- κ B cascade [6,7]. The NF- κ B pathway controls inflammation and oxidative stress [8]. Prolonged NF- κ B activation contributes to endothelial dysfunction, which then promotes pericyte loss [9,10]. As a result, hyperglycemia disrupts pericyte function and phenotype, impairing neovascularization [10,11]. Besides hyperglycemia, other factors like hyperlipidemia and low-grade inflammation also contribute

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to endothelial dysfunction [3,10]. The latter is characterized by increased fibrinogen, C-reactive protein, IL-6, IL-1, and TNF, which collectively activate the immune system [10,12]. Ultimately, the wound site becomes a complex biochemical environment marked by high glucose levels, oxidative stress, hypoxia, and, eventually, bacterial infection. These conditions hinder macrophages from shifting from an inflammatory to a pro-healing phenotype, thereby delaying proper wound resolution [1].

Photobiomodulation therapy (PBMT) is an increasingly recognized method for accelerating wound healing [13,14]. In our previous work, we showed that PBMT (5 J/cm²) applied to healthy mice attracted NG2⁺ pericytes and Nestin⁺ neural progenitors to the wound bed. Additionally, wounds treated with PBMT displayed consistent structural regeneration, closely resembling the original skin tissue [13]. In fact, PBMT promotes cell proliferation and angiogenesis in endothelial cells by stimulating vascular endothelial growth factor (VEGF) expression [15,16]. A classic placebo-controlled clinical trial demonstrated that PBMT (30 J/cm²) increased cutaneous microcirculation in patients with diabetic microangiopathy [17]. Earlier studies applying PBMT (4 and 6 J/cm²) to diabetic skin also reported significantly faster wound healing compared to untreated controls, mainly due to increased collagen production [18,19]. In streptozotocin-induced Type 1 diabetic rats, PBMT treatment (6.23 J/cm²) reduced the expression of NF- κ B and the Receptor for Advanced Glycation End-products (RAGE) in the peripheral nervous system, while suppressing TNF expression in the central nervous system [20]. Ultimately, through the p38 Mitogen-Activated Protein Kinases (MAPK) and NF- κ B-mediated signaling pathways, PBMT (11.46 J/cm²) protects vascular cells from inflammation-induced apoptosis [21].

Although several studies have explored PBMT in diabetic models [17–24], the mechanisms behind the accelerated wound repair in diabetes are still underexplored. Using a pericyte reporter mouse model (NestinGFP⁺NG2DsRed⁺), we examine the effect of PBMT on pericytes during wound healing in streptozotocin-induced diabetic Type 1 mice.

2. Material and methods

2.1. Animals

All experiments adhered to the ARRIVE guidelines. The NestinGFP⁺NG2DsRed⁺ transgenic phenotypes were obtained as previously described [13]. This model exhibits endogenous fluorescence for two proteins: NG2DsRed/pericytes (shown in red) and NestinGFP/neural progenitor cells (labeled in green). Double-labeled populations, NestinGFP⁺NG2DsRed⁺ (shown in yellow), can also be observed.

All animals were kept under a 12:12-h light-dark cycle and fed freely. Ten-week-old male or female NestinGFP⁺NG2DsRed⁺ or wild-type (WT) mice were used for molecular, histological, and confocal analyses. To determine the sample size, one of the most unstable pericyte-related variables was used: PDGFR- β expression, which has a coefficient of variation of 23% [25]. To ensure experimental reliability, a 25% confidence interval was used. Rounding the calculated *n* to the nearest whole number resulted in *n* = 5. Furthermore, one additional animal per experimental time point was included to prevent loss of statistical power in the event of any animal attrition or experimental losses, resulting in a final *n* = 6 per group. All experiments received approval from the Animal Care and Use Committee at the Universidade Federal de Minas Gerais (protocol #62/2019).

2.2. Streptozotocin-induced Type 1 diabetes (DM)

DM was chemically induced in mice as previously described [26]. Mice received daily intraperitoneal injections of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 50 mg/kg, diluted in 50 mM sodium citrate buffer, for five consecutive days. Two weeks after the final injection, body weight and blood glucose levels were measured at

24 h, 3 days, and 7 days to confirm the onset of diabetes. Glucose levels in the blood exceeding 300 mg/dL, along with clinical signs of polydipsia, polyphagia, and polyuria, were considered diabetic. Healthy animals (*n* = 3) were used as controls.

2.3. Experimental model validation

Blood samples were collected periodically via the caudal vein, and blood glucose levels were measured using the Accu-Check Active glucose monitor (Roche-Brasil, São Paulo, SP, Brazil). To assess the animals' average blood glucose over the past two weeks, a fructosamine test was performed on day 7. After anesthesia, blood was collected through puncture of the abdominal aorta. Following clotting at room temperature for 30 min, samples were centrifuged at 3000 rpm for 15 min. The serum was analyzed using the Cobas Mira Plus® (Roche-Brasil) with a fructosamine kit (Biotécnica, Varginha, MG, Brazil). Additionally, the expression of GLUT1 – a glucose transporter regulated by insulin – was measured by RT-qPCR and compared between STZ-induced Type 1 diabetic mice and healthy controls.

2.4. Wound healing model

Animals were anesthetized, and wounds were created twelve days after the last STZ injection. Full-thickness excisions were performed simultaneously on each side of the midline of the dorsal skin. Briefly, using a 4.0 mm biopsy punch, two circular wounds were made and bordered with a 0.5 mm silicone splint to prevent premature wound contraction (Fig. 1). The left wound was treated with PBMT, while the right served as the sham control.

2.5. Photobiomodulation (PBMT)

A customized diode laser, functioning in punctual, contact and continuous operation modes (indium–gallium–aluminum–phosphide/InGaAlP, 660 nm wavelength, 0.028 cm² spot area, 7 s, 5 J/cm², 0.71 W/cm², 20 mW, 0.14 J total energy per point) [13]. PBMT was applied transoperatively and continued daily for up to 7 days. A thick black cardboard paper was placed over the wound to prevent light exposure in the sham group. To ensure the reliability of our comparative analysis, the procedure was mimicked in the control group with the device turned off, maintaining identical experimental conditions across all groups.

2.6. Macroscopy

Under anesthesia, wound sizes were measured at 24 h, day 3, and day 7 using a caliper. Digital photographs of each wound were taken through day 7.

2.7. Laser doppler analysis

Local blood flow was measured at two specific time points (days 3 and 6 after irradiation) using a Laser Doppler imager (MoorLID2-IR). During the procedure, mice were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg, respectively, IP). We used the Moor LDI V5.3 software to analyze the scanned images; the average perfusion units were calculated from the mean pixel density of each wound area to indicate regional blood supply.

2.8. Histology

The mice were euthanized at three different times after wounding: 24 h, 3 days, and 7 days. Tissues were collected, leaving 2.0 mm beyond the wound borders, fixed in 4% paraformaldehyde (PFA) for 48 h, and embedded in paraffin. A microtome was used to cut the blocks into 5 μ m sections. Slides were then routinely stained. Five consecutive histological fields per sample were analyzed at 50 $\times$ magnification using an

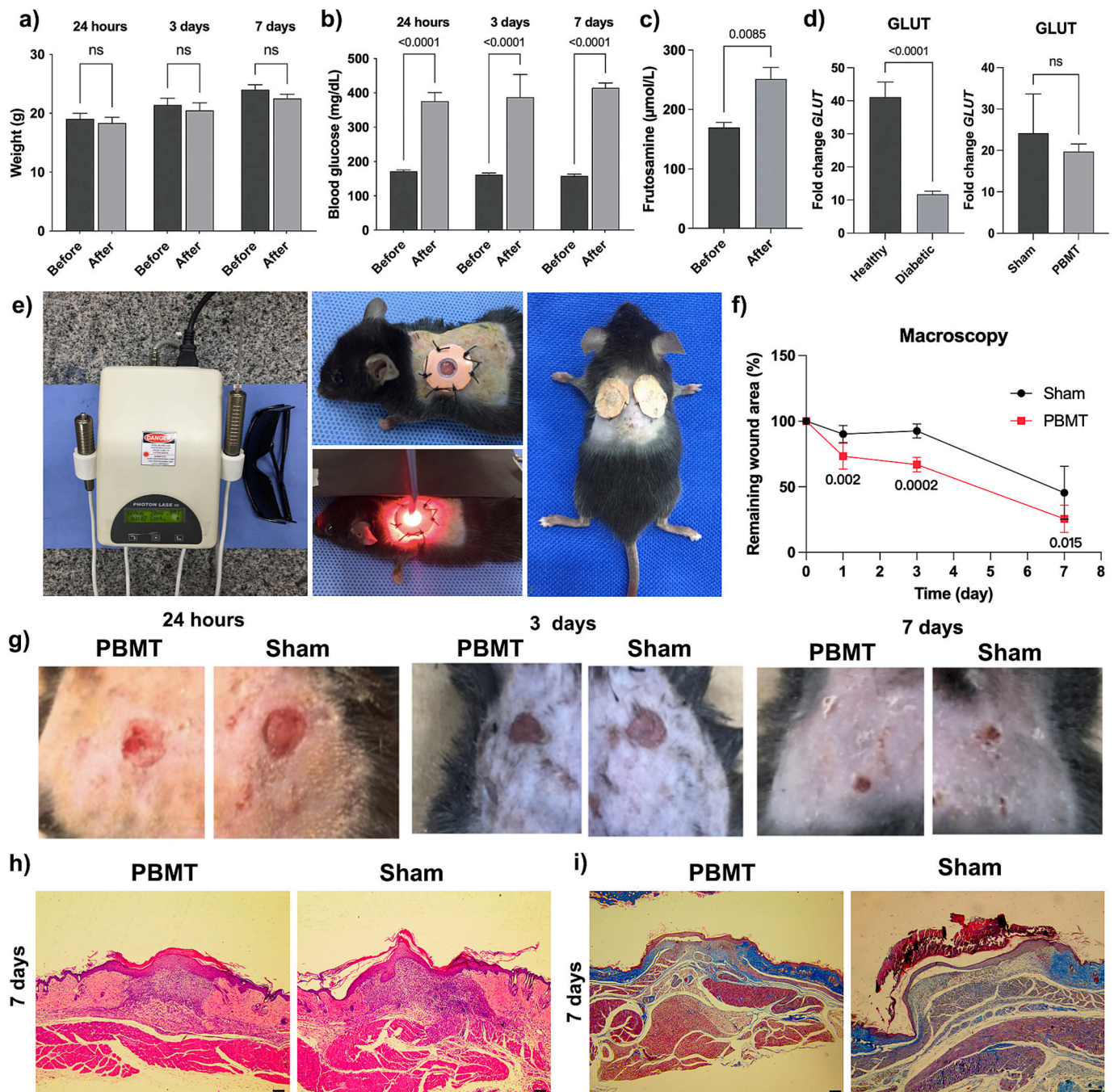


Fig. 1. Quantitative and qualitative in vivo analysis of diabetes parameters and remaining wound area in sham and PBMT-treated (5 J/cm^2) groups ($n = 6$). Bar graphs display animal body weight (a) and blood glucose levels (b) at 24 h, 3 days, and 7 days. Fructosamine levels at day 7 (c). Bar graphs compare GLUT1 fold change between healthy and diabetic mice, as well as between sham and PBMT-treated wounds at day 3 (d). Animal procedures involved applying PBMT trans-operatively and daily (e). A curve graph shows the remaining wound area between sham and PBMT-treated groups (f). Representative macroscopic images at 24 h, 3 days, and 7 days are shown in panel (g), while hematoxylin-eosin and Masson's trichrome-stained images at 7 days are displayed in panels (h) and (i), respectively. Notable findings include reduced wound contraction and granulation tissue formation, along with increased collagen production in PBMT-treated samples. PBMT = photobiomodulation therapy. GLUT = insulin-stimulated glucose transporter 1. NS = not significant. Magnification bar = $200 \mu\text{m}$.

optical microscope (Axioskop 40, Zeiss, Göttingen, Lower Saxony, Germany).

2.9. Masson's trichrome stain

Slides were immersed in Harris' hematoxylin for 40 s. Sections were then washed for two minutes and stained with Masson's trichrome for 17 min. Five regions of the wound borders and beds were photographed at $\times 50$ magnification using an optical microscope (Axioskop 40, Zeiss)

to evaluate collagen fibers.

2.10. Confocal analysis

Imaging of NestinGFP⁺NG2DsRed⁺ double-transgenic mice was performed using a confocal microscope (Eclipse Ti with a Zeiss LSM 880, Oberkochen, Germany). Samples were analyzed at 24 h, 3 days, and 7 days. Briefly, after fixation with 4% PFA, samples were immersed in 30% sucrose (w/v) overnight and embedded in OCT. Sections were cut at

25 μm thickness and mounted with DAPI counterstaining (Abcam, Cambridge, UK). A Plan Apo $\times 10$ objective was used. For NG2⁺Nestin⁺ (green/GFP) and NG2⁺Nestin⁺ (red/DsRed) quantification, each channel was analyzed separately. Before analysis, the threshold was adjusted, and the raw fluorescence in each image was quantified using ImageJ (NIH, Bethesda, Maryland, USA). NG2⁺Nestin⁺ pericytes exhibiting yellow fluorescence due to the colocalization of GFP and DsRed markers were quantified by the area using Image J (NIH).

2.11. Endothelin-1 (ET-1) immunostaining

Criosectioned wounds were permeabilized with 0.2% Triton X-100 and blocked using a universal blocker (ThermoFisher Scientific, Waltham, MA, USA) to prevent nonspecific binding. Primary rabbit polyclonal antibody against ET-1 (ThermoFisher Scientific) (1:100) was incubated for 1 h at room temperature. After several washes with PBS, sections were incubated with a secondary antibody (1:750, goat anti-rabbit Alexa Fluor™ 405, ThermoFisher Scientific). Images were captured at $\times 20$ magnification using a fluorescence microscope (Nexcope, Ningbo Yongxin Optics Co., Ningbo, China). The ET-1-immunostained area was quantified after manual threshold adjustment in ImageJ (NIH).

2.12. Diabetic skin cell culture

Intact skin cells from diabetic and healthy mice were isolated as previously described [27]. Cells were grown in regular medium (Dulbecco's Modified Eagle Medium, ThermoFisher), adding 10% fetal bovine serum, 100 UI/mL penicillin/streptomycin, and an additional 28 mM glucose solution [28] to simulate diabetic conditions in vitro (all from Gibco, ThermoFisher Scientific).

2.12.1. Colony-forming unit – Fibroblastic (CFU-F) counting

CFU-F ability was evaluated by plating 500 cells per mL into a six-well microtitration plate. After 2 weeks of culture, cells were fixed in 4% PFA for 30 min. CFU-F colonies were stained with 1% toluidine blue dye (Sigma-Aldrich) for 1 h. CFU-F were quantified using ImageJ software. Only colonies containing more than 50 cells were counted.

2.12.2. Cell viability/survival

Cell viability/survival was analyzed according to the manufacturer's instructions (MTT, Invitrogen, ThermoFisher Scientific). Cells were plated at 1×10^3 cells/well into 96-well microtitration plates in sextuplicate. Samples were analyzed at 24, 48, and 72 h. The 540 nm filter was used for reading.

2.12.3. Cell scratch assay

Cells grown in regular media were plated into 48-well microtitration plates at 2×10^4 cells/well in quadruplicate. After confluence, a “cell scratch” was performed using a micropipette tip (p200) along the wells' midline. The percentage of remaining wound area was calculated in ImageJ at 0, 12, 24, and 48 h. Images were acquired at $\times 40$ magnification using a Cytation 5 Cell Imaging system (Winooski, VT, USA).

2.12.4. Adipogenic differentiation

For adipogenic differentiation induction, 1×10^5 cells/well in 24-well microtitration plates were grown in α -MEM supplemented with 10% FBS, 100 U/mL penicillin-streptomycin, 2 mM L-glutamine, 100 μM ascorbic acid, 0.5 μM hydrocortisone, 0.5 mM 3-isobutyl-1-methylxanthine, 60 μM indomethacin, and 10 $\mu\text{g}/\text{mL}$ insulin. Regular or induction media were refreshed every 2–3 days.

2.12.5. Oil red staining

Lipid droplets were quantified using the Oil Red-O (ORO) assay. Point five ORO (w/v) was prepared in isopropanol and further diluted in a 6:4 isopropanol-to-water mixture. After 4% PFA for 30 min, cells were

stained with Oil Red O for 30 min. Images were captured at $400 \times$ magnification (Cytation 5 Cell Imaging). Lipid accumulation was assessed using ImageJ software.

2.12.6. Real time-quantitative polymerase chain reaction (RT-qPCR)

Samples were analyzed at day 3 for the in vivo experiments. In vitro, adipogenic differentiation was evaluated by measuring peroxisome proliferator-activated receptor γ (PPAR γ) at day 14. Cells cultivated in regular media for 14 days were also analyzed for expression of the neural-glial antigen 2 (NG2), Nestin, interleukin-1 α (IL-1 α), and interleukin-1 receptor antagonist (IL-1RA) genes. cDNA was produced from 2500 ng of total RNA (SuperScript VILO IV Master Mix, ThermoFisher Scientific).

Table 1 lists the quantified primer sequences. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. The $2^{-\Delta\Delta\text{CT}}$ method was used to calculate gene expression. In mouse experiments, intact diabetic skin served as the reference sample (1-fold). Cells cultivated in regular media and not irradiated were the reference sample for the in vitro assays.

2.13. Statistical analysis

Statistical significance between groups was analyzed after data normalization and further assessed using an unpaired Student's *t*-test with Welch's correction in GraphPad Prism (GraphPad Prism Version 9.0c for Mac, GraphPad Software, La Jolla, California, USA). Multiple comparisons, where applicable, were performed using Analysis of Variance (ANOVA) followed by the Bonferroni post hoc. Data are presented as mean $\pm$ standard error of the mean (SEM). The confidence level was set at 5%.

3. Results

3.1. Glycemic bioassays confirmed DM status

Fig. 1 shows no change in body weight, increased blood glucose ($p < 0.0001$), and higher fructosamine levels after DM induction ($p < 0.01$) (Fig. 1a-c). GLUT1 - a glucose transporter regulated by insulin - was

Table 1
Primer sequences used for RT-qPCR analysis.

Gene	Primers
<i>Nestin</i>	Forward: 5'-TGGCTACATACAGGACTCTGC-3' Reverse: 5'-AAGGATGTTGGGCTGAGGAC-3'
<i>NG2</i>	Forward: 5'-CTGTTCTCACACAGAGGAGCC-3' Reverse: 5'-TGGACAGACGGTCAACTTCC-3'
<i>IL-1RA</i>	Forward: 5'-AACCAGTCATTGCTGGTACTTA-3' Reverse: 5'-GCCCAAGAACACACTATGAAGGTC-3'
<i>IL-1α</i>	Forward: 5'-ACGTCAAGCAACGGGAAGAT-3' Reverse: 5'-AAGGTGCTGATCTGGGTGG-3'
<i>GFAP</i>	Forward: 5'-CTAACGACTATCGCCGCCAA-3' Reverse: 5'-CTCTAGGGACTCGTTCGTGC-3'
<i>ITGA3</i>	Forward: 5'-AGGCCTCGCTCAGCTTAATG-3' Reverse: 5'-GCAGTTTTCATGGCAGCCTC-3'
<i>NFkB</i>	Forward: 5'-CCACTGTCAACAGATGGCCC-3' Reverse: 5'-AGCGGAATCGAAATCCCTC-3'
<i>TNF-α</i>	Forward: 5'-GATCGGTCCCCAAGGGATG-3' Reverse: 5'-TGAGGCTCTGGGCCATAGAA-3'
<i>PPARγ</i>	Forward: 5'-GTCACACTCTGACAGGAGCC-3' Reverse: 5'-CACCCTCTCTTCAAATCTTGT-3'
<i>GLUT1</i>	Forward: 5'-GGATCCCAGCAGCAAGAAGGT-3' Reverse: 5'-CGAAGCTGAGTATCCGAGC-3'
<i>GAPDH</i>	Forward: 5'-ACGGCCGATCTTCTTGTC-3' Reverse: 5'-CGCCAAATCCGTTACACCGA-3'

NG2 = Neural/glial antigen 2; IL-1RA = Interleukin 1 receptor antagonist; IL-1 α = Interleukin 1 α ; GFAP = glial fibrillary acidic protein; ITGA3 = Integrin alpha 3; NF-kB = Nuclear Factor Kappa B; TNF = Tumor Necrosis Factor; PPAR γ = Peroxisome proliferator-activated receptor γ ; GLUT1 = insulin-stimulated glucose transporter 1; GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

significantly reduced in the diabetic group compared to the healthy one ($p < 0.0001$) (Fig. 1d). When evaluating the sham and PBMT-treated wounds, GLUT1 expression remained statistically similar between groups (Fig. 1d).

3.2. PBMT enhances wound size reduction

Macroscopic analysis showed that the remaining wound area was significantly reduced in the groups treated with PBMT compared to the control at all experimental times (Fig. 1e-g) ($p < 0.05$). Routine histological analysis indicated less inflammatory response in the PBMT-treated group. Masson's trichrome also revealed greater collagen deposition compared to the control (Fig. 1h and i).

3.3. PBMT promotes an influx of $NG2^+nestin^-$, $NG2^-Nestin^+$ and $NG2^+nestin^+$ cells at the wound bed

Fig. 2 shows bright-field and epifluorescent images of PBMT-treated wounds and sham wounds at 24 h, 3 days, and 7 days after the procedure. At 24 h, there were no noticeable differences between the groups (Fig. 2a). However, at 3 days post-procedure, $NG2^+Nestin^-$, $NG2^-Nestin^+$, and $NG2^+Nestin^+$ cells appeared in the wound bed, especially at the wound edges (Fig. 2b). The number of $NG2^+Nestin^+$ cells was significantly higher in the PBMT-treated group compared to the sham group at day 3 ($p < 0.05$) (Fig. 3a), but there was no significant

difference at day 7 ($p > 0.05$) (Fig. 3b).

At 7 days, more $NG2^+Nestin^-$ and $NG2^-Nestin^+$ cells appeared at the wound bed and margins of PBMT-treated wounds compared to the sham ones ($p < 0.05$) (Fig. 4a).

3.4. Pro-resolutive signals are activated in PBMT-treated groups without changes in the *in vivo* pro-inflammatory scenario

The gene expression profile at day 3 (Fig. 4b-i) showed that Nestin mRNA levels were higher following PBMT compared to the control, although not statistically significant ($p > 0.05$). Additionally, NG2 and GFAP, a glial cell marker, mRNA were upregulated ($p < 0.05$ and $p < 0.01$, respectively), as well as IL1RA, a key molecule for reducing IL-1 expression ($p < 0.05$). Levels of IL-1 α , TNF, and NF- κ B were similar between sham and PBMT-treated wounds.

3.5. Blood perfusion was similarly stimulated by PBMT and sham treatments, but a larger blood vessel lumen area was observed after irradiation

Fig. 5 displays the distribution of pericyte-covered blood vessels in sham or PBMT-treated wounds (Fig. 5a). Doppler analysis showed that neither treatment differed from the other, but both increased blood flow within the wounds compared to intact skin at day 3 ($p < 0.05$) (Fig. 5b). Notably, when compared to intact skin, the perfusion difference was

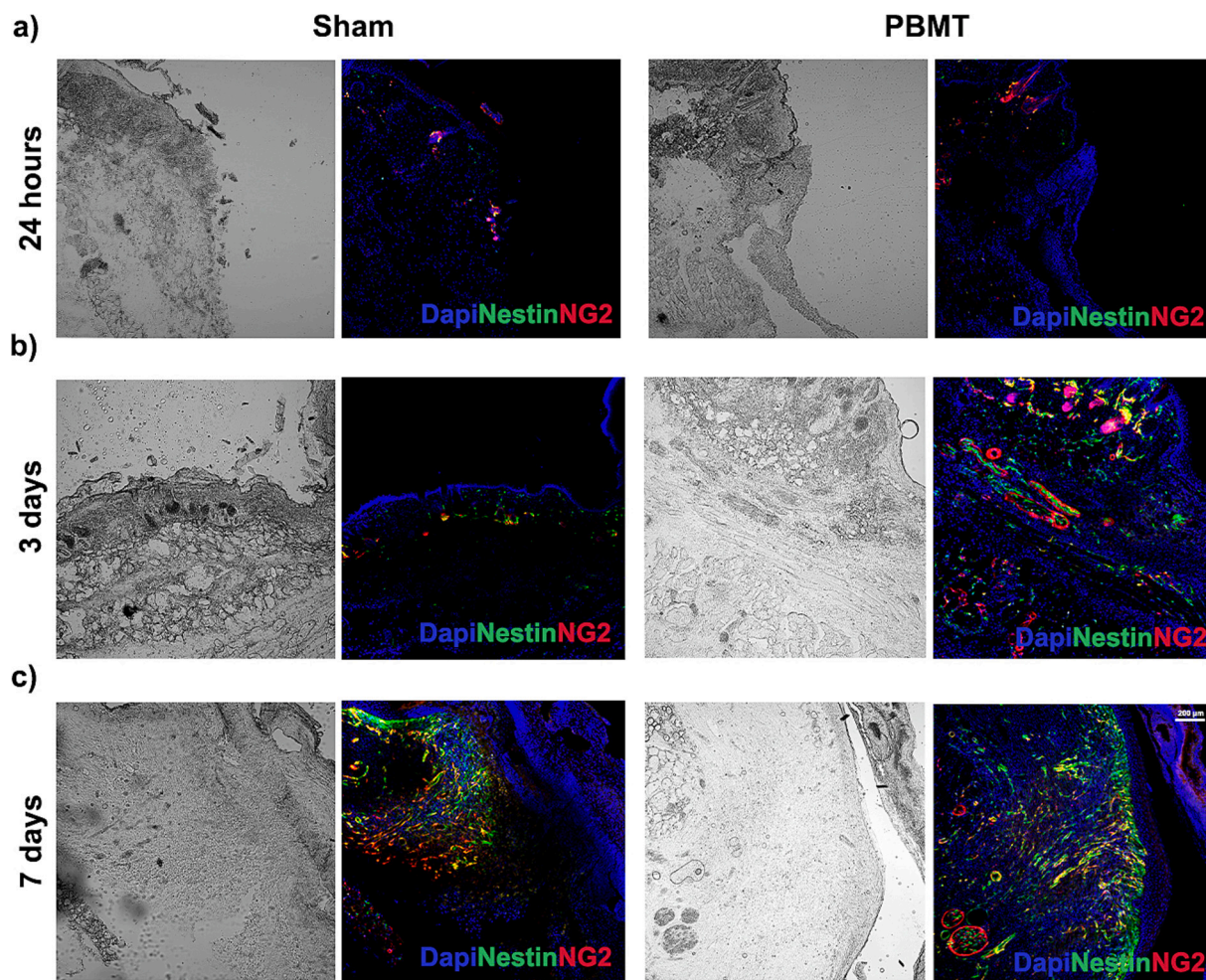


Fig. 2. Representative bright-field and epifluorescent images of the wound edges showing endogenous fluorescence for $NG2^+$ (marked in red) and $Nestin^+$ (marked in green) at 24 h (a), day 3 (b) and 7 (c) in the sham and PBMT-treated (5 J/cm^2) samples. Blood vessels structures covered by pericytes are also stained in red. PBMT = photobiomodulation therapy. Magnification bar = 200 μm . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

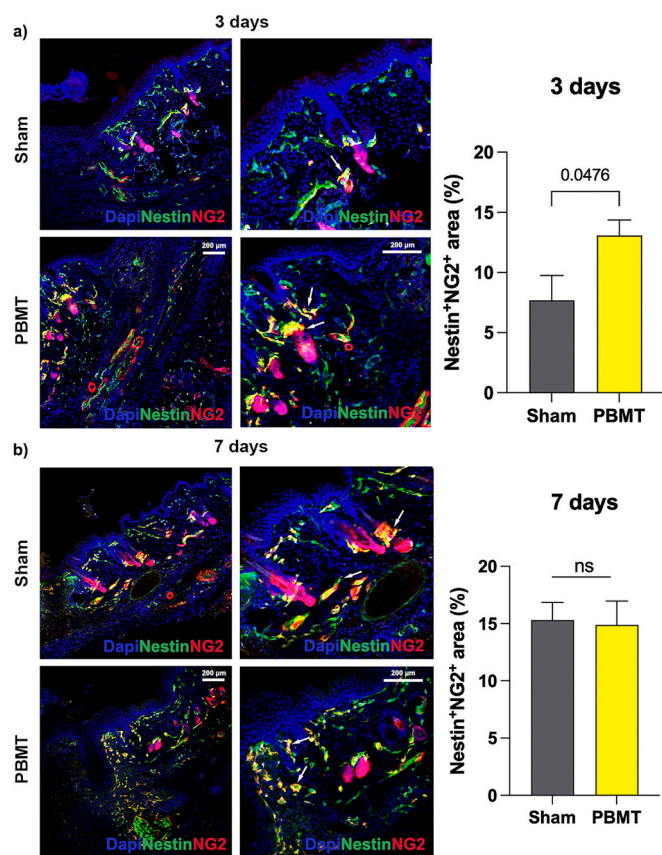


Fig. 3. Representative epifluorescent images of the wound edges showing NG2⁺Nestin⁺ cells marked in yellow (arrows) at 3 days (a) and 7 days (b) in the sham and PBMT-treated (5 J/cm²) samples. Bar graphs display the in vivo quantification of NG2⁺Nestin⁺ cells at 3 days (a) and 7 days (b) in both the sham and PBMT-treated (5 J/cm²) groups. PBMT = photobiomodulation therapy. NS = not significant. Magnification bar = 200 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

greater with PBMT treatment than with sham (Fig. 5b). By day 6, there were no differences between groups or when compared to the intact skin ($p > 0.05$) (Fig. 5b).

A morphological analysis of the lumen area of pericyte-covered blood vessels was performed on the sham and PBMT-treated samples (Fig. 5c). On day 3, there was no significant difference between the groups. However, on day 7, the lumen area was larger in PBMT-treated wounds ($p < 0.05$) (Fig. 5c).

The assessment of ET-1 expression, a potent endogenous vasoconstrictor, showed no difference between groups, although there was a numerical reduction in ET-1 expression in PBMT-treated wounds ($p > 0.05$) (Fig. 6). (See Fig. 7.)

3.6. PBMT promoted binary effects in vitro

The DM status in vitro was confirmed by lower GLUT1 expression in diabetic cells compared to healthy cells ($p < 0.001$) (Fig. 4a). PBMT significantly increased GLUT1 expression in healthy samples ($p < 0.01$). In contrast, no statistical difference was observed in diabetic cells (Fig. 4a). CFU-F showed no difference between PBMT-treated and sham groups (Fig. 4b). Cell viability decreased at 24 and 72 h after PBMT but increased at 48 h ($p < 0.05$). At 7 days, there was no statistical difference in cell viability between groups (Fig. 4c). The cell scratch bioassay showed increased cell migration following PBMT at 12 h but a significant reduction at 24 h ($p < 0.001$) (Fig. 4d and e). At 48 h, no difference in the remaining wound area was observed between the PBMT-treated

and sham groups, with all groups showing complete wound closure (Fig. 4d).

Under induction, adipogenic cell differentiation was stimulated by PBMT, leading to increased expression of PPAR γ mRNA and lipid droplet content ($p < 0.05$) (Fig. 4f). After 2 weeks of cell culture, NG2 and Nestin genes were significantly upregulated following PBMT ($p < 0.05$). IL1a was also markedly upregulated, while IL1RA was downregulated in the PBMT-treated groups ($p < 0.05$).

4. Discussion

In diabetes, microvascular complications are marked early by pericyte loss [10]. Dysfunctional pericytes contribute to disease progression by promoting vasoconstriction and thickening of the basement membrane through excessive deposition of fibronectin and collagen, ultimately causing tissue ischaemia [10,29]. Our findings suggest that PBMT may enhance the lumen of pericyte-covered vessels and improve the flow of perivascular cells in diabetic wound beds, potentially restoring pericyte coverage during wound healing. An increase in neural progenitors at the injury site was also observed. Notably, PBMT significantly increased IL-1 receptor antagonist (IL-1RA) expression in vivo, despite promoted only modest changes in the overall inflammatory scenario. An increase in adipogenic differentiation and NG2/Nestin expression in vitro was also induced by light.

The cooperation between pericytes and endothelial cells is crucial in both the formation and long-term stability of the vasculature [30]. Diabetes is associated with vascular complications characterized by disrupted pericyte-EC communication, leading to pericyte loss and abnormal endothelial cell behavior [10]. In our study, PBMT promoted a significant influx of NG2+ cells, including double NG2⁺Nestin⁺ perivascular cells at day 3, which are considered less lineage-committed cellular components [13]. Both in vivo and in vitro, NG2 expression was upregulated in PBMT-treated samples. Aligning with our findings, previous studies have reported that PBMT can mobilize perivascular cells to sites of injury, thereby supporting faster and more coordinated tissue repair in healthy subjects [13,31–33].

Notably, morphometric analysis showed that PBMT not only enhanced pericyte recruitment but also increased the luminal areas of pericyte-covered vessels at day 7. These structural improvements suggest a move toward a more mature and stable microvascular network, even though significant changes in total perfusion (Doppler analysis) were not observed at this time. To support these findings, we also examined endothelin-1 (ET-1), a potent vasoconstrictor often elevated in the diabetic microvasculature, which hampers perfusion and delays healing [34]. The trend toward ET-1 downregulation may indicate that PBMT helps reduce the vasospastic state in diabetic wounds, even if full hemodynamic recovery, as measured by Doppler, takes longer to become apparent. A previous study corroborates our findings showing that PBMT reduces smooth muscle cell reactivity in arteries, and this phenomenon is accompanied by decreased ET-1 expression [35]. Further studies are necessary to investigate the microvascular functional integrity of PBMT in this Type 1 diabetic model.

Glial cells provide structural support and regulate nerve regeneration and tissue homeostasis [36]. During wound healing, Schwann cells may co-locate with pro-resolutive M2 macrophages [37]. In diabetic mice, Schwann and glial cells exhibit an impaired ability to quickly initiate repair processes following skin wound injury, demonstrating limitations in cell dedifferentiation, cell-cycle re-entry, and cell migration [38,39]. Here, neural progenitor cells (NG2⁺Nestin⁺) and Nestin mRNA levels increased at the injury site. We also observed significant upregulation of glial fibrillary acidic protein (GFAP) in vivo in PBMT-treated wounds. Glial cells in peripheral nerve tissues are mainly activated and play a key role in promoting wound repair [27]. A study on diabetic neuropathic ulcers in rodents showed that PBMT (at 4, 6, and 8 J/cm²) improved wound oxidative balance by modulating antioxidant and reactive oxygen species levels [40]. Other reports have also highlighted the positive

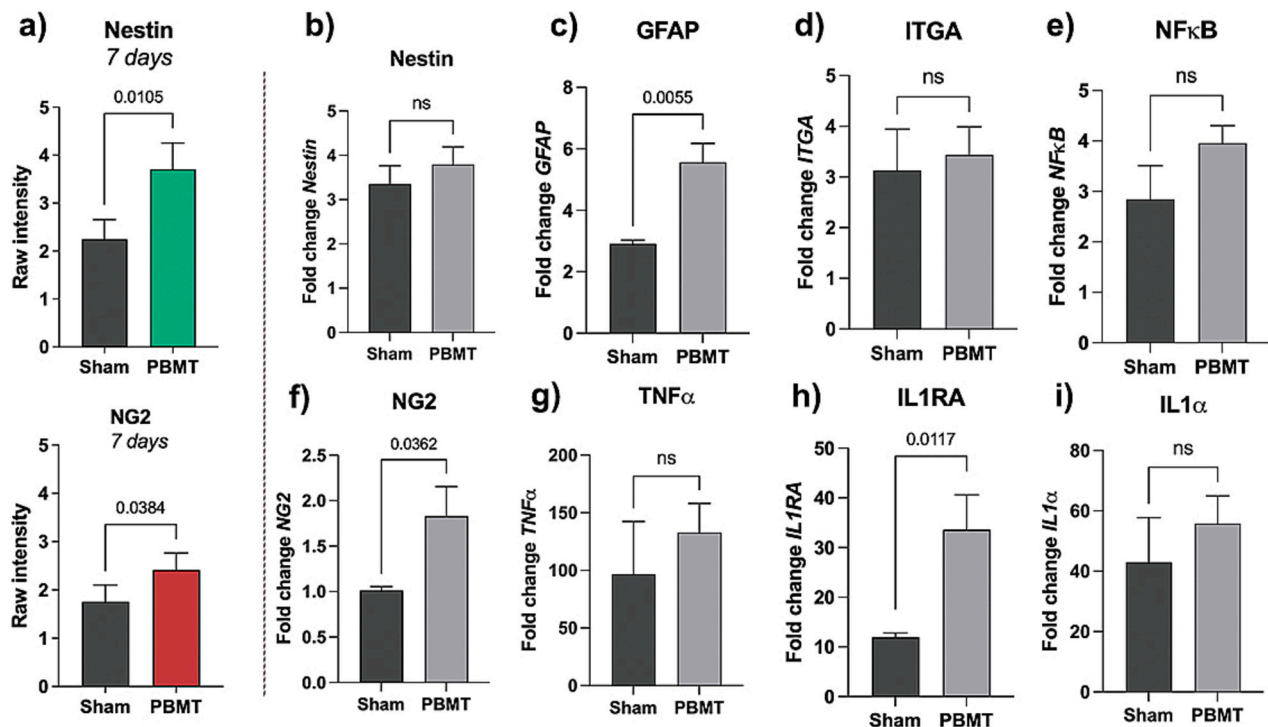


Fig. 4. Bar graphs showing in vivo quantification of NG2 and Nestin endogenous fluorescence in cells from sham and wounds treated with PBMT (5 J/cm²) at day 7 (a). The bar graphs display expression levels of Nestin (b), GFAP = glial fibrillary acidic protein (c), *ITGA3* = integrin alpha 3 (d), *NF-κB* = nuclear factor kappa B (e), *NG2* = neural/glial antigen 2 (f), *TNF-α* = tumor necrosis factor-alpha (g), *IL-1RA* = interleukin 1 receptor antagonist (h), and *IL-1α* = interleukin 1 alpha (i). PBMT = photobiomodulation therapy. NS = not significant. Magnification bar = 200 μm.

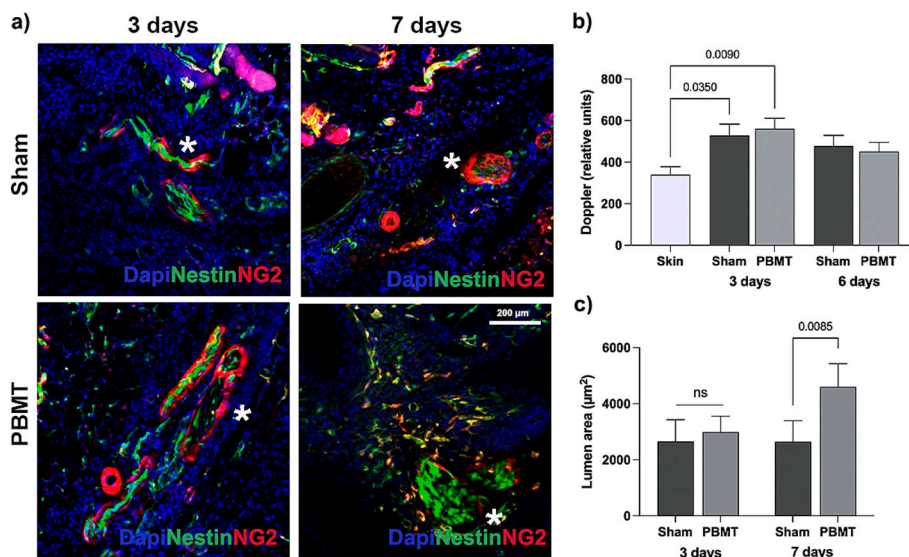


Fig. 5. Representative epifluorescent images of the wound edges showing pericyte-covered vessels (asterisks) in sham and wounds treated with PBMT (5 J/cm²) at days 3 and 7 (a). Bar graph showing Laser Doppler analysis at days 3 and 6 comparing both groups and intact skin. Bar graph displays pericyte-covered vessels lumen area quantification (c). PBMT = photobiomodulation therapy. NS = not significant. Magnification bar = 200 μm.

role of PBMT on glial cells and nerve regeneration [41,42]. Future research into the spatial and temporal roles of glial cells in PBMT-treated diabetic individuals, especially regarding protective glial responses, is warranted.

In vivo, we observed that NF-κB and TNF mRNA levels were comparable between PBMT-treated and non-irradiated samples at day 3. In fact, NF-κB represents the prototypical pathway that drives pro-inflammatory signaling and its activation is primarily triggered by

TNF cascades. [1]. A study analyzing IL-1β, IL-6, and TNF, reported that pro-inflammatory cytokines were significantly reduced following PBMT in diabetic animals. RAGE analysis did not show any differences among groups, whereas NF-κB p65 showed higher concentrations in STZ samples than in STZ + PBMT samples [43]. Conversely, another study reported upregulation of NF-κB, TNF, MMP, and S100a8 in PBMT-treated skin wound samples (800 nm; 20, 40, and 60 J/cm²) [44]. Despite an initially inflammatory state induced by PBMT, irradiated samples

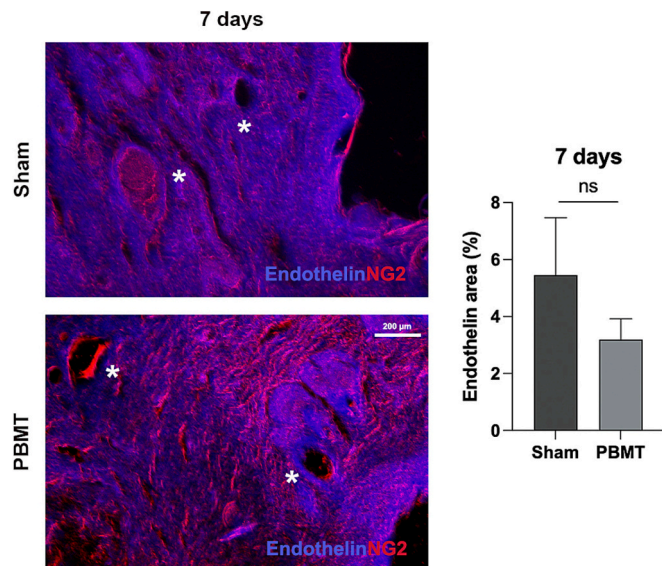


Fig. 6. Representative epifluorescent images of endothelin 1 (ET-1) expression in the sham and PBMT-treated wounds on day 7. Bar graph depicting ET-1 quantification by area. PBMT = photobiomodulation therapy. NS = not significant. Asterisks indicate structures suggestive of vessels. **Magnification bar = 200 μ m.**

showed increased dermal expression of type I and III procollagen at day 30. Accordingly, the literature reports conflicting findings on NF- κ B signaling pathway activation following PBMT [12,45,46], corroborating the notion that PBMT-generated ROS can be tightly regulated. This might indicate that, despite PBMT promoting modest changes in the overall inflammatory microenvironment, accelerated wound healing was still observed.

In our study, diabetic cells showed reduced viability and limited migration in response to PBMT at specific time points, unlike a previous study that used 5 J/cm² [47]. Considering IL-1 α and IL-1RA expression, we observed that an inflammatory profile persisted even after 14 days in culture. In vivo, PBMT did not alter the expression of IL-1 α or integrin-related genes. However, IL-1RA was upregulated considerably following photobiomodulation. IL-1RA is known to dampen IL-1 α and IL-1 β activity, contributing to inflammatory control and regeneration [48]. A study observed that PBMT reduced gingival inflammation by down-regulating IL-1 β and TNF cytokines, and upregulating IL-1RA, whose neutralization partially reversed the healing effects of light. Previously, we also found that PBMT significantly increased IL-1RA expression during epithelial-mesenchymal crosstalk [49,50]. The divergence between our in vivo and in vitro findings regarding the IL-1 α /IL-1RA ratio likely reflects the inherent limitations of isolated cell cultures [49]. This is especially relevant for the inflammatory panel, given its labile expression during the initial phase of tissue repair and the prolonged cell culture context, which leads to late-stage cellular stress [51]. These results underscore that the regenerative effects of PBMT are best captured within the complex physiological context of the wound bed, where multi-cellular signaling pathways are intact.

The STZ-induced Type 1 diabetes mouse model was confirmed by elevated blood sugar and fructosamine levels, along with decreased expression of insulin-stimulated glucose transporter 1 (GLUT1). However, we saw progressive wound healing after photobiomodulation. Although PBMT did not increase GLUT1 levels in vivo or in vitro, diabetic cells showed improved adipogenic differentiation and higher PPAR γ levels compared to controls. Recently, we found that PBMT promotes NG2 activation via lipolysis in fat cells during wound healing, along with increased PPAR γ expression [31]. The enhanced adipogenic signature observed in the PBMT group likely represents a regenerative advantage by expanding the pool of progenitor cells available for tissue

repair. An earlier in vitro study also showed that PBMT boosts PPAR γ activity in insulin-resistant fat cells via the Dok1/ERK pathway. This process prevents excessive lipolysis, reducing circulating free fatty acids, and suggests that PBMT could be a useful treatment for lipid metabolism issues in insulin resistance [52].

The choice of 660 nm (red spectrum) over near-infrared (NIR, e.g., 808 nm) is justified by its optimal absorption by cytochrome c oxidase in superficial injuries [53]. Unlike NIR, which has greater tissue penetration for deep structures (muscle/bone), 660 nm is the primary indication for cutaneous wound healing, where the target cells, such as keratinocytes, fibroblasts, and the superficial NG2⁺ pericytes investigated here, are located within the first few millimeters of the tissue [32,54]. The selection of the 660 nm wavelength and the specific irradiation protocol was based on extensive evidence from our research group, which has successfully utilized these parameters for superficial tissue repair and inflammatory modulation, including on perivascular and neural components of the skin [13,31,32,49,50].

Our study limitations include using the same animal in both the control and test groups, which was necessary due to the rarity of the transgenic mouse model. While some literature suggests potential systemic anti-inflammatory effects of PBMT [55], the significant differences we observed in pericyte recruitment and luminal area between the treated and untreated sides within the same animal indicate that the primary regenerative effects were locally driven. Additionally, although we included both male and female mice to ensure biological representativeness, the sample size was powered to evaluate a primary effect of a pericyte marker rather than to detect subtle sex-dependent differences in healing kinetics; these nuances remain a factor for future large-scale investigations.

5. Conclusion

Our data support increased pericyte-covered vessel lumen and increased pericyte flow during wound repair following PBMT. Neural cellular components were also increased at the injury site. The pro-resolving cytokine IL-1RA was significantly upregulated, while pro-inflammatory markers remained essentially unchanged. These findings suggest that PBMT may enhance wound healing by promoting resolution-oriented pathways, even in the presence of persistent chronic inflammation within the diabetic wound microenvironment.

Bioethical approvals

Experiments were approved by the UFMG Ethical Committee (#62/2019).

CRediT authorship contribution statement

Rafaela Ferrer de Oliveira: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Humberto Jácome-Santos:** Writing – review & editing, Writing – original draft, Formal analysis. **Sara Santos Bernardes:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Luiza Almeida Queiroz Ferreira:** Writing – original draft, Methodology, Investigation, Formal analysis. **Talyta Couto de Freitas:** Writing – original draft, Methodology, Investigation, Formal analysis. **Matheus Henrique de Souza Silva:** Writing – original draft, Methodology, Investigation, Formal analysis. **Fabiola de Oliveira Paes Leme:** Writing – original draft, Resources, Formal analysis. **Luciola da Silva Barcelos:** Writing – original draft, Resources, Formal analysis. **Marina Gonçalves Diniz:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Gleide Fernandes Avelar:** Writing – original draft, Resources, Formal analysis, Data curation. **Ricardo Alves Mesquita:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization. **Alexander Birbrair:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding

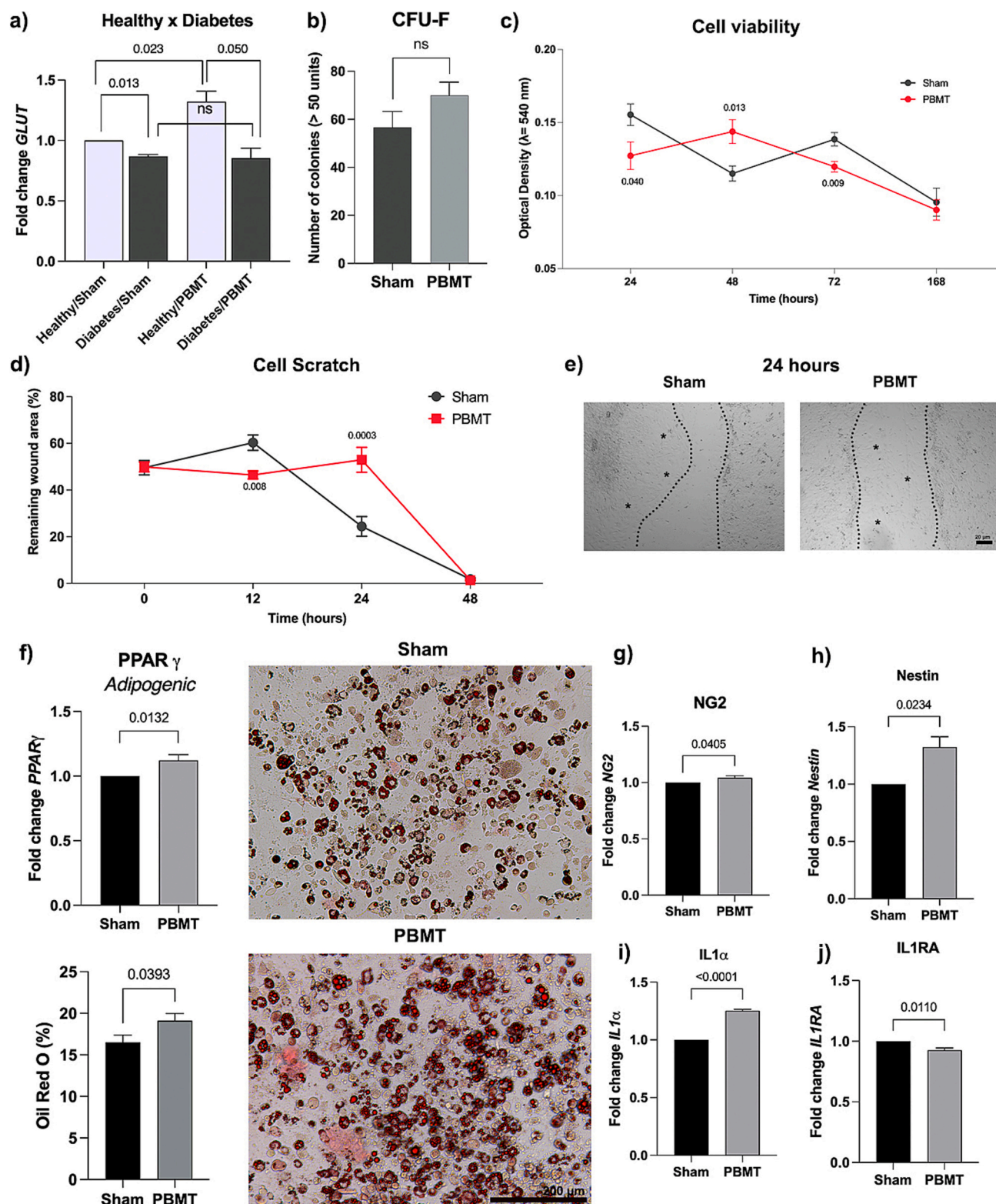


Fig. 7. Diabetic skin assays. Bar graph comparing *GLUT1* expression between healthy and diabetic cells, with or without PBMT treatment (5 J/cm²) (a). Bar graph showing colony-forming units - fibroblastic (CFU-F) comparison between sham and PBMT-treated groups (b). Curves displaying cell viability at 24, 48, 72, and 168 h for each group (c). Curve from a scratch assay comparing cell migration at 12, 24, and 48 h between sham and PBMT-treated groups (d). Representative bright-field images showing cell migration at 24 h, with asterisks indicating evenly distributed cells in the wound (e). Fold change in the expression of peroxisome proliferator-activated receptor (PPAR γ) and lipid droplet quantification in monolayers grown in adipogenic induction media (f). Oil Red O - stained lipid droplets (f). Gene expression of neural-glial antigen 2 (NG2) (g), Nestin (h), interleukin 1- α (IL-1 α) (i), and interleukin 1- α receptor antagonist (IL-1RA) (j) in monolayers cultured in regular media. PBMT = photobiomodulation therapy. PPAR γ = peroxisome proliferator-activated receptor gamma; GLUT1 = insulin-stimulated glucose transporter 1; GAPDH = Glyceraldehyde-3-phosphate dehydrogenase. Magnification bar = 200 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

acquisition. **Manoela Domingues Martins:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. **Ivana Márcia Alves Diniz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly's linguistic analysis to improve the readability and language of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

Authors declare no competing financial interests or personal relationships that may have influenced the elaboration of this manuscript.

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Data availability

Data will be made available on request.

References

- [1] H.X. Teh, S.J. Phang, M.L. Looi, U.R. Kuppusamy, B. Arumugam, Molecular pathways of NF- κ B and NLRP3 inflammasome as potential targets in the treatment of inflammation in diabetic wounds: a review, *Life Sci.* 334 (2023) 122228, <https://doi.org/10.1016/j.lfs.2023.122228>.
- [2] C.J. Nolan, N.B. Ruderman, S.E. Kahn, O. Pedersen, M. Prentki, Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes, *Diabetes* 64 (3) (2015) 673–686, <https://doi.org/10.2337/db14-0694>.
- [3] N. Di Pietrantonio, I. Cappellacci, D. Mandatori, M.P.A. Baldassarre, A. Pandolfi, C. Pipino, Role of epigenetics and metabolomics in predicting endothelial dysfunction in type 2 diabetes, *Adv. Biol. (Weinh.)* 7 (9) (2023) e2300172, <https://doi.org/10.1002/adbi.202300172>.
- [4] Y. Zhang, J. Liu, I.J. de Souza Araujo, L. Bahammam, L.L. Munn, G.T.J. Huang, Neovascularization by DPSC-ECs in a tube model for pulp regeneration study, *J. Dent. Res.* 103 (6) (2024) 652–661, <https://doi.org/10.1177/00220345241236392>.
- [5] P. Li, H. Fan, Pericyte loss in diseases, *Cells* 12 (15) (2023) 1931, <https://doi.org/10.3390/cells12151931>.
- [6] J.A. Beckman, A.B. Goldfine, M.B. Gordon, M.A. Creager, Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans, *Circulation* 103 (12) (2001) 1618–1623, <https://doi.org/10.1161/01.cir.103.12.1618>.
- [7] P. Di Tomo, R. Canali, D. Ciavardelli, S. Di Silvestre, A. De Marco, A. Giardinelli, C. Pipino, N. Di Pietro, F. Virgili, A. Pandolfi, B-Carotene and lycopene affect endothelial response to TNF- α reducing nitro-oxidative stress and interaction with monocytes, *Mol. Nutr. Food Res.* 56 (2) (2012) 217–227, <https://doi.org/10.1002/mnfr.201100500>.
- [8] F. Cai, W. Chen, R. Zhao, Y. Liu, Mechanisms of Nrf2 and NF- κ B pathways in diabetic wound and potential treatment strategies, *Mol. Biol. Rep.* 50 (6) (2023) 5355–5367, <https://doi.org/10.1007/s11033-023-08392-7>.
- [9] J. Zhao, S. Yang, B. Shu, L. Chen, R. Yang, Y. Xu, J. Xie, X. Liu, S. Qi, Transient high glucose causes persistent vascular dysfunction and delayed wound healing by the DNMT1-mediated Ang-1/NF- κ B pathway, *J. Invest. Dermatol.* 141 (6) (2021) 1573–1584, <https://doi.org/10.1016/j.jid.2020.10.023>.
- [10] N. Warmke, K.J. Griffin, R.M. Cubbon, Pericytes in diabetes-associated vascular disease, *J. Diabetes Complicat.* 30 (8) (2016) 1643–1650, <https://doi.org/10.1016/j.jdiacomp.2016.08.005>.
- [11] U.A. Okonkwo, L. Chen, D. Ma, V.A. Haywood, M. Barakat, N. Urao, L.A. DiPietro, Compromised angiogenesis and vascular integrity in impaired diabetic wound healing, *PLoS One* 15 (4) (2020) e0231962, <https://doi.org/10.1371/journal.pone.0231962>.
- [12] Shurtz-Swirski R, Sela S, Herskovits AT, Shasha SM, Shapiro G, Nasser L, Kristal B. Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. *Diabetes Care* 2001;24(1):104–10. doi: <https://doi.org/10.2337/diacare.24.1.104>.
- [13] I.B. do Valle, P.H.D.M. Prazeres, R.A. Mesquita, T.A. Silva, H.M. de Castro Oliveira, P.R. Castro, I.D.P. Freitas, S.R. Oliveira, N.A. Gomes, R.F. de Oliveira, L. F. Marquiere, S. Macari, F.A. do Amaral, H. Jácome-Santos, L.S. Barcelos, G. B. Menezes, M.M. Marques, A. Birbrair, I.M.A. Diniz, Photobiomodulation drives pericyte mobilization towards skin regeneration, *Sci. Rep.* 10 (1) (2020) 19257, <https://doi.org/10.1038/s41598-020-76243-7>.
- [14] T.N. Mgwenya, H. Abrahamse, N.N. Hourel, Photobiomodulation studies on diabetic wound healing: an insight into the inflammatory pathway in diabetic wound healing, *Wound Repair Regen.* 33 (1) (2025) e13239, <https://doi.org/10.1111/wrr.13239>.
- [15] K. Góralczyk, J. Szymańska, M. Łukowicz, E. Dreła, R. Kotzbach, M. Dubiel, M. Michalska, B. Góralczyk, A. Zając, D. Roś, Effect of LLLT on endothelial cells culture, *Lasers Med. Sci.* 30 (1) (2015) 273–278, <https://doi.org/10.1007/s10103-014-1650-0>.
- [16] V. Cury, A.I. Moretti, L. Assis, P. Bossini, S. Crusca Jde, C.B. Neto, R. Fangel, H. P. de Souza, M.R. Hamblin, N.A. Parizotto, Low level laser therapy increases angiogenesis in a model of ischemic skin flap in rats mediated by VEGF, HIF-1 α and MMP-2, *J. Photochem. Photobiol. B* 125 (2013) 164–170, <https://doi.org/10.1016/j.jphotobiol.2013.06.004>.
- [17] A. Schindl, M. Schindl, H. Schön, R. Knobler, L. Havelec, L. Schindl, Low-intensity laser irradiation improves skin circulation in patients with diabetic microangiopathy, *Diabetes Care* 21 (4) (1998) 580–584, <https://doi.org/10.2337/diacare.21.4.580>.
- [18] G.A. Maiya, P. Kumar, L. Rao, Effect of low intensity helium-neon (He-ne) laser irradiation on diabetic wound healing dynamics, *Photomed. Laser Surg.* 23 (2) (2005) 187–190, <https://doi.org/10.1089/pho.2005.23.187>.
- [19] J. de Alencar Fonseca Santos, M.B.D. Campelo, R.A. de Oliveira, R.A. Nicolau, V.E. A. Rezende, E.A.L. Arisawa, Effects of low-power light therapy on the tissue repair process of chronic wounds in diabetic feet, *Photomed. Laser Surg.* 36 (6) (2018) 298–304, <https://doi.org/10.1089/pho.2018.4455>.
- [20] N.L. Ferreira, I.R.C. Rocha, M. Chacur, Unraveling the RAGE-NF- κ B pathway: implications for modulating inflammation in diabetic neuropathy through photobiomodulation therapy, *Lasers Med. Sci.* 39 (1) (2024) 222, <https://doi.org/10.1007/s10103-024-04171-3>.
- [21] Y.H. Chu, S.Y. Chen, Y.L. Hsieh, Y.H. Teng, Y.J. Cheng, Low-level laser therapy prevents endothelial cells from TNF- α /cycloheximide-induced apoptosis, *Lasers Med. Sci.* 33 (2) (2018) 279–286, <https://doi.org/10.1007/s10103-017-2364-x>.
- [22] A.T. Santomauro, G. Boden, M.E. Silva, D.M. Rocha, R.F. Santos, M.J. Ursich, P. G. Strassmann, B.L. Wajchenberg, Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects, *Diabetes* 48 (9) (1999) 1836–1841, <https://doi.org/10.2337/diabetes.48.9.1836>.
- [23] L. Gong, Z. Zou, L. Liu, S. Guo, D. Xing, Photobiomodulation therapy ameliorates hyperglycemia and insulin resistance by activating cytochrome c oxidase-mediated protein kinase B in muscle, *Aging (Albany NY)* 13 (7) (2021) 10015–10033, <https://doi.org/10.18632/aging.202760>.
- [24] S. Guo, L. Gong, Q. Shen, D. Xing, Photobiomodulation reduces hepatic lipogenesis and enhances insulin sensitivity through activation of CaMKK β /AMPK signaling pathway, *J. Photochem. Photobiol. B* 213 (2020) 112075, <https://doi.org/10.1016/j.jphotobiol.2020.112075>.
- [25] R.J. Konrad, I. Mikolaenko, J.F. Tolar, K. Liu, J.E. Kudlow, The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic beta-cell O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase, *Biochem. J.* 356 (Pt 1) (2001) 31–41, <https://doi.org/10.1042/0264-6021:3560031>.
- [26] J. Kyyriäinen, X. Ekolle Ndode-Ekane, A. Pitkänen, Dynamics of PDGFR β expression in different cell types after brain injury, *Glia* 65 (2) (2017) 322–341, <https://doi.org/10.1002/glia.23094>.
- [27] V. Parfejevs, J. Debbache, O. Shakhova, S.M. Schaefer, M. Glausch, M. Wegner, U. Suter, U. Riekstina, S. Werner, L. Sommer, Injury-activated glial cells promote wound healing of the adult skin in mice, *Nat. Commun.* 9 (1) (2018) 236, <https://doi.org/10.1038/s41467-017-01488-2>.
- [28] S. Tarallo, E. Beltramo, E. Berrone, M. Porta, Human pericyte-endothelial cell interactions in co-culture models mimicking the diabetic retinal microvascular environment, *Acta Diabetol.* 49 (Suppl. 1) (2012) S141–S151, <https://doi.org/10.1007/s00592-012-0390-5>.
- [29] M. Isono, S. Chen, S.W. Hong, M.C. Iglesias-de la Cruz, F.N. Ziyadeh, Smad pathway is activated in the diabetic mouse kidney and Smad3 mediates TGF- β -induced fibronectin in mesangial cells, *Biochem. Biophys. Res. Commun.* 296 (5) (2002) 1356–1365, [https://doi.org/10.1016/s0006-291x\(02\)02084-3](https://doi.org/10.1016/s0006-291x(02)02084-3).
- [30] M. Castro, M. Potente, The blood-brain barrier-a metabolic ecosystem, *EMBO J.* 41 (9) (2022) e111189, <https://doi.org/10.15252/embj.2022111189>.

- [31] N.A. Gomes, I.B. do Valle, F.O. Gleber-Netto, T.A. Silva, Oliveira HMC, R.F. de Oliveira, Ferreira LAQ, L.S. Castilho, Reis PHRG, Prazeres PHDM, G.B. Menezes, C. S. de Magalhães, R.A. Mesquita, M.M. Marques, A. Birbrair, Diniz IMA, Nestin and NG2 transgenes reveal two populations of perivascular cells stimulated by photobiomodulation, *J. Cell. Physiol.* 237 (4) (2022) 2198–2210, <https://doi.org/10.1002/jcp.30680>.
- [32] Diniz IMA, R.F. de Oliveira, I.B. do Valle, C.C. Picoli, H. Jácome-Santos, L. de Almeida Queiroz Ferreira, G.F. Avelar, M.G. Diniz, A. Birbrair, Photobiomodulation therapy induces NG2 activation through dermal adipocyte lipolysis during wound healing, *J. Photochem. Photobiol. B* 267 (2025) 113151, <https://doi.org/10.1016/j.jphotobiol.2025.113151>.
- [33] L. Anita, M.J. Choi, G.N. Yin, J. Ock, M.H. Kwon, S.S. Hong, J.H. Kang, J.K. Suh, J. K. Ryu, Photobiomodulation therapy for diabetic erectile dysfunction targeting neuroinflammation and neurovascular regeneration, *Sci. Rep.* 15 (1) (2025) 20513, <https://doi.org/10.1038/s41598-025-04873-w>.
- [34] H.A. Bull, C.B. Bunker, G. Terenghi, D.R. Springall, Y. Zhao, J.M. Polak, P. M. Dowd, Endothelin-1 in human skin: immunolocalization, receptor binding, mRNA expression, and effects on cutaneous microvascular endothelial cells, *J. Invest. Dermatol.* 97 (4) (1991) 618–623, <https://doi.org/10.1111/1523-1747.ep12483000>.
- [35] M. Mackiewicz-Milewska, E. Grześ, A.C. Kroszczyński, M. Cisowska-Adamak, H. Mackiewicz-Nartowicz, L. Baran, I. Szymkuć-Bukowska, M. Wiciński, W. Hagner, G. Grześ, The influence of low level laser irradiation on vascular reactivity, *Adv. Med. Sci.* 63 (1) (2018) 64–67, <https://doi.org/10.1016/j.advms.2017.06.002>.
- [36] D.A. Padovani-Claudio, C.J. Ramos, M.E. Capozzi, J.S. Penn, Elucidating glial responses to products of diabetes-associated systemic dyshomeostasis, *Prog. Retin. Eye Res.* 94 (2023) 101151, <https://doi.org/10.1016/j.preteyeres.2022.101151>.
- [37] N. Yoshida, N. Edanami, N. Ohkura, T. Maekawa, N. Takahashi, A. Tohma, K. Izumi, T. Maeda, A. Hosoya, H. Nakamura, K. Tabeta, Y. Noiri, K. Yoshida, M2 phenotype macrophages colocalize with schwann cells in human dental pulp, *J. Dent. Res.* 99 (3) (2020) 329–338, <https://doi.org/10.1177/0022034519894957>.
- [38] M.Y. Ou, P.C. Tan, Y. Xie, K. Liu, Y.M. Gao, X.S. Yang, S.B. Zhou, Q.F. Li, Dedifferentiated Schwann cell-derived TGF-β3 is essential for the neural system to promote wound healing, *Theranostics* 12 (12) (2022) 5470–5487, <https://doi.org/10.7150/thno.72317>.
- [39] S. Zhou, L. Wan, X. Liu, D. Hu, F. Lu, X. Chen, F. Liang, Diminished schwann cell repair responses play a role in delayed diabetes-associated wound healing, *Front. Physiol.* 13 (2022) 814754, <https://doi.org/10.3389/fphys.2022.814754>.
- [40] G. Karkada, G.A. Maiya, P. Arany, M.R. Kg, S. Adiga, S.U. Kamath, Dose-response relationship of photobiomodulation therapy and oxidative stress markers in healing dynamics of diabetic neuropathic ulcers in Wistar rats, *J. Diabetes Metab. Disord.* 22 (1) (2022) 393–400, <https://doi.org/10.1007/s40200-022-01157-2>.
- [41] Y. Ma, M. Yang, X. Chen, W. Qu, X. Qu, P. He, The effectiveness of photobiomodulation therapy on inferior alveolar nerve injury: A systematic review and META-analysis, *PLoS One* 18 (8) (2023) e0287833, <https://doi.org/10.1371/journal.pone.0287833>.
- [42] B. Li, X. Wang, Photobiomodulation enhances facial nerve regeneration via activation of PI3K/Akt signaling pathway-mediated antioxidant response, *Lasers Med. Sci.* 37 (2) (2022) 993–1006, <https://doi.org/10.1007/s10103-021-03344-8>.
- [43] M.T. Goova, J. Li, T. Kislinger, W. Qu, Y. Lu, L.G. Bucciarelli, S. Nowygrod, B. M. Wolf, X. Caliste, S.F. Yan, D.M. Stern, A.M. Schmidt, Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice, *Am. J. Pathol.* 159 (2) (2001) 513–525, [https://doi.org/10.1016/S0002-9440\(10\)61723-3](https://doi.org/10.1016/S0002-9440(10)61723-3).
- [44] P. Avci, T.T. Nyame, G.K. Gupta, M. Sadasivam, M.R. Hamblin, Low-level laser therapy for fat layer reduction: a comprehensive review, *Lasers Surg. Med.* 45 (6) (2013) 349–357, <https://doi.org/10.1002/lsm.22153>.
- [45] C.Y. Fukuoka, H.P. Vicari, C.R. Sipert, U.K. Bhawal, Y. Abiko, V.E. Arana-Chavez, A. Simões, Early effect of laser irradiation in signaling pathways of diabetic rat submandibular salivary glands, *PLoS One* 15 (8) (2020) e0236727, <https://doi.org/10.1371/journal.pone.0236727>.
- [46] A. de Farias Gabriel, V.P. Wagner, C. Correa, L.P. Webber, E.F.S. Pilar, M. Curra, V. C. Carrard, M.A.T. Martins, M.D. Martins, Photobiomodulation therapy modulates epigenetic events and NF-κB expression in oral epithelial wound healing, *Lasers Med. Sci.* 34 (7) (2019) 1465–1472, <https://doi.org/10.1007/s10103-019-02745-0>.
- [47] S.M. Ayuk, N.N. Hourel, H. Abrahamse, Effect of 660 nm visible red light on cell proliferation and viability in diabetic models in vitro under stressed conditions, *Lasers Med. Sci.* 33 (5) (2018) 1085–1093, <https://doi.org/10.1007/s10103-017-2432-2>.
- [48] X. Kou, X. Xu, C. Chen, M.L. Sanmillan, T. Cai, Y. Zhou, C. Giraudo, A. Le, S. Shi, The Fas/Fap-1/Cav-1 complex regulates IL-1RA secretion in mesenchymal stem cells to accelerate wound healing, *Sci. Transl. Med.* 10 (432) (2018) eaai8524, <https://doi.org/10.1126/scitranslmed.aai8524>.
- [49] R.F. Oliveira, L.F. Marquiere, C.B.S. Gomes, P.T.R. de Abreu, L.A.Q. Ferreira, L. A. Diniz, N.A. Gomes, H. Jácome-Santos, A. Moreno, S. Macari, R.A. Mesquita, T. A. Silva, M.M. Marques, I.M.A. Diniz, Interplay between epithelial and mesenchymal cells unveils essential proinflammatory and pro-resolutive mediators modulated by photobiomodulation therapy at 660 nm, *Wound Repair Regen.* 30 (3) (2022) 345–356, <https://doi.org/10.1111/wrr.13010>.
- [50] L. Fassarella Marquiere, R. Ferrer Oliveira, L. Almeida Queiroz Ferreira, N. A. Gomes, M. Martins Olavo de Souza, B. Gomes Lucardians, J.V. Reis Cruvinel, A. L. Morais Perdigão, R. Alves Mesquita, T.A. Silva, S. Santos Bernardes, M. Gonçalves Diniz, I.M. Alves Diniz, Oral mucosa cues for regeneration using photobiomodulation, *Lasers Med. Sci.* 40 (1) (2025) 97, <https://doi.org/10.1007/s10103-025-04343-9>.
- [51] M.J. Han, W.J. Lee, J. Choi, Y.J. Hong, S.J. Uhm, Y. Choi, J.T. Do, Inhibition of neural stem cell aging through the transient induction of reprogramming factors, *J. Comp. Neurol.* 529 (3) (2021) 595–604, <https://doi.org/10.1002/cne.24967>.
- [52] X. Jiang, L. Huang, D. Xing, Photoactivation of Dok1/ERK/PPARγ signaling axis inhibits excessive lipolysis in insulin-resistant adipocytes, *Cell. Signal.* 27 (7) (2015) 1265–1275, <https://doi.org/10.1016/j.cellsig.2015.03.010>.
- [53] I. Selestin Raja, C. Kim, N. Oh, J.H. Park, S.W. Hong, M.S. Kang, C. Mao, D.W. Han, Tailoring photobiomodulation to enhance tissue regeneration, *Biomaterials* 309 (2024) 122623, <https://doi.org/10.1016/j.biomaterials.2024.122623>.
- [54] K. Kwiczen, A. Zegar, J. Jung, P. Brzoza, M. Kwitniewski, U. Godlewska, B. Grygier, P. Kwiecinska, A. Morytko, J. Cichy, Architecture of antimicrobial skin defense, *Cytokine Growth Factor Rev.* 49 (2019) 70–84, <https://doi.org/10.1016/j.cytogfr.2019.08.001>.
- [55] T.G. da Silva, R.S. Ribeiro, A.L. Mencalha, Fonseca A. de Souza, Photobiomodulation at molecular, cellular, and systemic levels, *Lasers Med. Sci.* 38 (1) (2023) 136, <https://doi.org/10.1007/s10103-023-03801-6>.