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ORIGINAL ARTICLE

The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients—a prospective, double-blind randomized study

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Abstract Chronic wounds, especially in diabetic patients, represent a challenging health issue. Since standard treatment protocols often do not provide satisfactory results, additional treatment methods—like phototherapy using low-level light therapy—are being investigated. The aim of our study was to evaluate the effect of phototherapy with light-emitting diodes on chronic wound treatment in diabetic and non-diabetic patients. Since a sufficient blood supply is mandatory for wound healing, the evaluation of microcirculation in the healthy skin at a wound's edge was the main outcome measure. Forty non-diabetic patients and 39 diabetics with lower limb chronic wounds who were referred to the University Medical Center Ljubljana between October 2012 and June 2014 were randomized to the treated and control groups. The treated group received phototherapy with LED 2.4 J/cm^2 (wavelengths 625, 660, 850 nm) three times a week for 8 weeks, and the control group received phototherapy with broadband 580–900 nm and power density 0.72 J/cm^2. Microcirculation was measured using laser Doppler. A significant increase in blood flow was noted in the treated group of diabetic and non-diabetic patients ($p = 0.040$ and $p = 0.033$), while there was no difference in the control groups. Additional Falanga wound bed score evaluation showed a significant improvement in both treated groups as compared to the control group. According to our results, phototherapy with LED was shown to be an effective additional treatment method for chronic wounds in diabetic and non-diabetic patients.

Keywords Low-level light therapy · LED · Microcirculation · Chronic wound

Introduction

Chronic wounds—wounds that do not heal in months or even years—are one of the most persisting medical challenges because of their vast influence on public health [1, 2]. Standard treatment approaches including debridement of the necrotic tissue, maintenance of a moist wound bed, and control of the infection often do not produce the desired result. Wounds in patients with diabetes mellitus represent an even bigger problem since the healing process in these patients is known to be impaired [1].

Therefore, additional treatment options such as negative pressure dressings, hyperbaric oxygen therapy, topical application of carbon dioxide, and light therapy [3] are often employed. Light therapy has increasingly been investigated ever since Mester incidentally discovered that low-level laser therapy (LLLT) accelerated hair regrowth in laser-irradiated rats [4].

The effect of LLLT was first investigated in vitro to verify the influence of LLLT on cell proliferation [5–7]. Fibroblasts in cell cultures that were stimulated with LLLT proliferated significantly faster compared to sham-irradiated control cells [8, 9]. Other cell lines (gingival and mucosal fibroblasts,

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keratinocytes, osteoblasts, etc.) also showed faster proliferation if treated with LLLT [10, 11]. The next step in investigating LLLT was in vivo experiments on animals (mostly rats), which showed faster wound healing following LLLT [8, 10, 12]. The encouraging results of preclinical studies prompted the introduction of LLLT to different fields of medicine (wound healing, rheumatology, oral and sports medicine, etc.) [13–16].

A recent survey critically reviewed eight clinical studies investigating the influence of LLLT on the healing of diabetic foot ulcers; all of the reviewed studies confirmed a beneficial effect of LLLT on the healing of diabetic ulcers [17].

Conversely, studies presenting data on LLLT and wound healing in general did not give such convincing conclusions. In his review in 2008, Sobanko concluded that LLLT in humans does not improve wound healing and advised better controlled studies in humans to determine the appropriate laser parameters and treatment protocol [18]. Kilik, on the other hand, confirmed that LLLT improved wound healing in normal and diabetic rats [1].

Wound healing in diabetic patients is probably impaired due to hyperglycemia, inhibition of inflammatory response, poor angiogenesis, fibroplasia and defects in collagen deposition, and differentiation of the extracellular matrix [1, 5].

The exact mechanism of low-power laser effect on tissue healing is not yet completely understood.

Studies have shown that LLLT accelerates the respiratory chain and increases reactive oxygen species (ROS), NO, and intracellular Ca^{2+} in stressed and hypoxic cells, but not in healthy cells [5, 19, 20]. Is it possible that the beneficial effect of LLLT on the wound-healing process in diabetic patients is more pronounced compared to non-diabetic patients because their cells are additionally hypoxic and stressed due to the diabetes itself?

The process of wound healing goes through the phases of inflammation, proliferation, and maturation [11]. A sufficient blood supply is mandatory for wound healing, but it is impaired in diabetic and non-diabetic patients with chronic wounds. This should be kept in mind when interpreting the results of published clinical studies.

The term LLLT was used for laser light only until the National Aeronautics and Space Administration (NASA) developed a new generation of light-emitting diodes (LEDs) to accelerate plant growth during space flights [21, 22]. Accelerated wound healing in astronauts treated with LED encouraged its use for medical purposes, and clinical experiences showed comparable results to LLLT [2, 21–23]. The abbreviation LLLT was later used for "low-level light therapy," including low-level laser therapy or low-level light therapy using LED.

New generations of LED proved to be effective in wound healing if the right wavelengths, power density, and doses were used [24, 25]. In his review, Chaves compared the efficacy of low-level light therapy with laser and LED and concluded that both yielded similar biological effects, with no significant differences [2]. Light from lasers is coherent while light from LED is not; however, according to Karu, coherence is lost during the interaction of light with biological tissue and thus is not a prerequisite for the process of photostimulation or photoinhibition [26].

Results from previous studies evaluating the effect of LLLT on chronic wound healing in general are conflicting. In diabetic patients, LLLT was predominantly shown to be effective, whereas in non-diabetic patients, its benefits were not as pronounced. The aim of our study was to compare the influence of LLLT (using LED) as an additional therapy for chronic wound healing in diabetic and non-diabetic patients. Since a sufficient blood supply to the wound area is mandatory for healing, the microcirculation of the healthy skin on the wound margin was the main outcome measure of our study. Additionally, the wound bed score according to Falanga was evaluated [27].

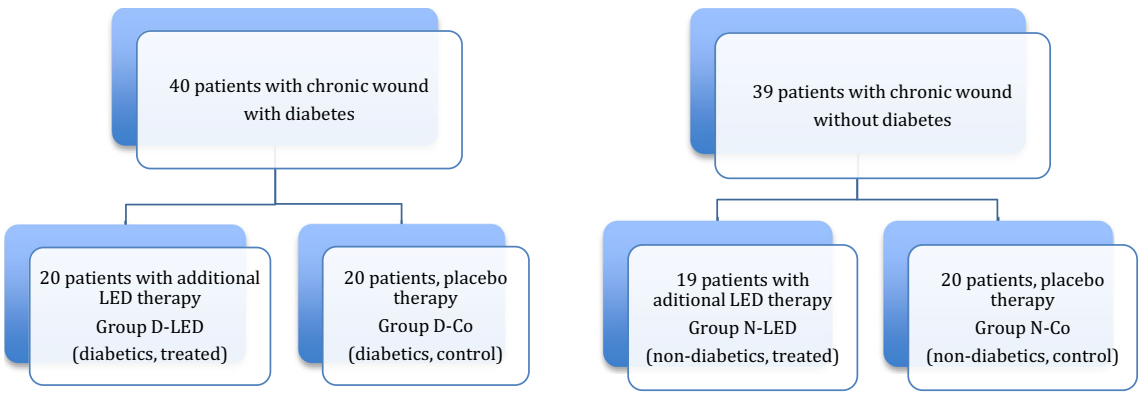

Fig. 1 Distribution and randomization of patients with below-knee chronic wounds

Table 1 Treatment regimes of active LED and placebo therapy

	LED wavelengths (nm)	Total energy density (J/cm^2) (time = 5 min)
Groups D-LED and N-LED (active)	625[a], 660[b], 850[c]	2.4 J/cm^2 (24%[a], 71%[b], 5%[c])
Groups D-Co and N-Co (placebo)	Broadband 580–900	0.72 J/cm^2

[a, b, c] Represent the contributing ratio of power density of corresponding wavelength

Materials and methods

Patients

Eighty patients with chronic wound below the knee, with or without diabetes mellitus, who were referred to the University Medical Center Ljubljana between October 2012 and June 2014, were included in the study.

Patients were divided into diabetic and non-diabetic groups according to the presence of diabetes mellitus and further randomized into treated and non-treated subgroups (Fig. 1). Exclusion criteria included patients whose wound surface was too large (over 15 cm × 20 cm) or patients with wounds expanding to several planes where even distribution of the light at irradiation could not be guaranteed.

One non-diabetic patient from the actively treated group failed to complete all applications of LED treatment due to personal reasons and was therefore excluded from the study.

All patients included in our study were referred to our clinic after their general practitioners failed to achieve satisfactory wound healing. The standard care provided by GPs includes taking care of the wound with wound dressings and occasionally with debridement of the necrotic tissue and antibiotic therapy. After examination, patients who met the inclusion criteria for our study immediately started with the standardized protocol.

All patients were treated according to common principles applied to the management of chronic wounds, including debridement of necrotic tissue, maintenance of a moist wound bed, and control of the infection.

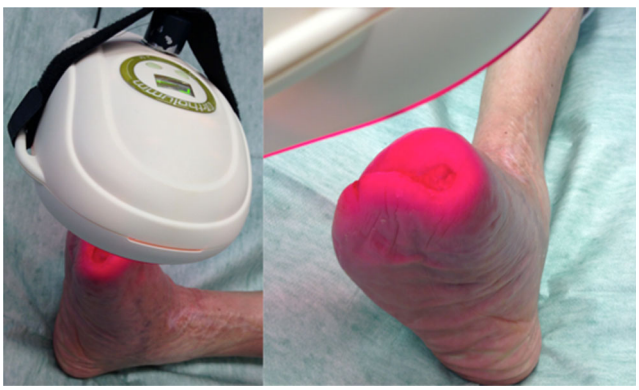

Fig. 2 Ortholumm was used as a LED source (light source surface was approximately 88 cm^2) at a distance of 10 cm, three times a week for 5 min. Treatment was performed for 8 weeks or, in the case of early healing, until wound closure

Additionally, both treated groups received active therapy with LED and both control groups received therapy with light that simulated LED, but had no known biological effect (placebo). The study was double-blind.

LED therapy

The source of light therapy in our study was a LED, and not laser as in most previously mentioned studies.

The treated groups (D-LED and N-LED) received active therapy with LED (Ortholumm, Votan, Slovenia), and the control groups (D-Co and N-Co) received therapy with light that simulated LED—placebo (Table 1).

Actively LED-treated groups were irradiated with a mixture of three wavelengths. The contributing power density of each wavelength is shown in percentages in Table 1. The LED source was a square wave modulated at a frequency in the kilohertz range, with a 50% duty cycle.

Placebo groups were irradiated with broadband spectrum (automobile light bulbs were built into the same LED housing and red filters were added) with the same 5-min exposure time. In the placebo device, total energy is equally distributed between wavelengths 580 and 900 nm. This means that the energy of every wavelength is approximately 0.00225 J/cm^2, which is 50 to 100 times less compared to the total energy densities in the active LED device. Therefore, we considered this to be placebo therapy.

The distance between the light source (LED or placebo) and the wound was 10 cm for all groups (Fig. 2).

Blood flow and Falanga wound bed evaluation

Blood analysis and microcirculation were evaluated using laser Doppler flow (LD flux) before the first treatment and at the end of the study.

Table 2 Falanga wound bed evaluation score [27]

Falanga score	Granulation	Fibrinous	Eschar
A	100%	–	–
B	50–100%	+	–
C	<50%	+	–
D	Any amount	+	+

Table 3 Group description—basic data

	Group D-LED ($n = 20$)	Group D-Co ($n = 20$)	Group N-LED ($n = 19$)	Group N-Co ($n = 20$)
Male/female	17/3	14/6	13/6	16/4
Age (mean ± SD)	61.15 ± 8.77	65.45 ± 9.57	63.84 ± 16.34	62.8 ± 11.88
BMI (mean ± SD)	30.72 ± 5.45	29.30 ± 4.65[a]	28.15 ± 5.65	26.58 ± 3.67[a]
Wound persistence in months (mean ± SD)	8.1 ± 6.13	9.15 ± 10.72	9.58 ± 16.76	9.4 ± 16.35
Wound surface in mm^2 (mean ± SD)	842 ± 74.22	978.21 ± 222.38	912.5 ± 110.89	814 ± 120.01

BMI body mass index

Only significant differences for $p < 0.05$ are shown: [a] $p = 0.04$

Microcirculation was measured on the intact skin at wound border using laser Doppler (LD) flux sensors (Angled probe 401, Perimed, Järfälla, Sweden) together with laser light sources at 780 nm (PF 4001 and PF 4002 Satelite, Perimed, Järfälla, Sweden).

Patients were scheduled for wound management and LED/placebo treatment three times a week. Wound status according to Falanga wound bed score (Table 2) was evaluated before the first treatment and every 2 weeks.

Statistical analysis

For statistical analysis, a paired *T* test or chi-square test was performed to compare the variables before and after treatment and between groups. The mean differences and 95% confidence intervals (95% CI) were calculated with two-sided probability (*p*) values. Significance level was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics, v. 19 (IBM Corp, Armonk, NY).

Results

Age and sex distribution as well as wound surface and wound persistence in months were comparable in all four subgroups (Table 3). There were some expected differences between diabetic and non-diabetic patients: diabetic patients had higher BMI, higher fasting glucose levels, higher levels of CRP, and lower hemoglobin values (Table 4).

Blood flow measured with LD flux revealed significantly increased microcirculation in LED-treated groups and no difference in control groups (Fig. 3).

Falanga wound bed evaluation showed significantly faster granulation and healing of the wound bed in both LED-treated groups compared to control groups (Fig. 4).

Blood analysis after 8 weeks of treatment showed no difference in fasting glucose levels, fibrinogen, hemoglobin, and SR in any of the groups. Figure 5 shows wounds of two patients from LED-treated groups.

Discussion

Wound healing is a complex process involving inflammation, proliferation, and maturation of the newly formed tissue [3, 28]. Wounds normally heal in 6–8 weeks or, in cases of larger or deeper wounds, they at least start healing by that time. If the process of healing is interrupted or impaired due to an infection or other causes (poor vascularization, malnutrition, diabetes, etc.), the wound does not heal and it becomes a chronic wound [28].

A standard approach to chronic wound treatment includes debridement of the necrotic tissue, use of wound dressings that maintain a moist wound bed, and control of the infection. Chronic wounds, however, are predominantly infected. In

Table 4 Group description—main blood analysis results before wound treatment

	Group D-LED ($n = 20$)	Group D-Co ($n = 20$)	Group N-LED ($n = 19$)	Group N-Co ($n = 20$)	*p* value
Fasting glucose level (mean ± SD)	7.8 ± 4.5[a]	8.86 ± 4.0[b]	5.6 ± 0.8[a]	5.6 ± 2.0[b]	[a]0.032 [b]0.003
Elevated CRP	13/20[c]	11/20	6/19[c]	9/20	[c]0.001
Hemoglobin (mean ± SD)	127.1 ± 13.9[d]	126.8 ± 14.9[e]	140.7 ± 16.5[d]	144.3 ± 14.1[e]	[d]0.008 [e]0.001
Fibrinogen (mean ± SD)	4.63 ± 1.04	4.67 ± 1.57	3.97 ± 1.05	4.11 ± 1.11	

CRP C-reactive protein

Only significant differences for $p < 0.05$ are shown

[a, b, c, d, e] Represent the *p* value of the compared corresponding values

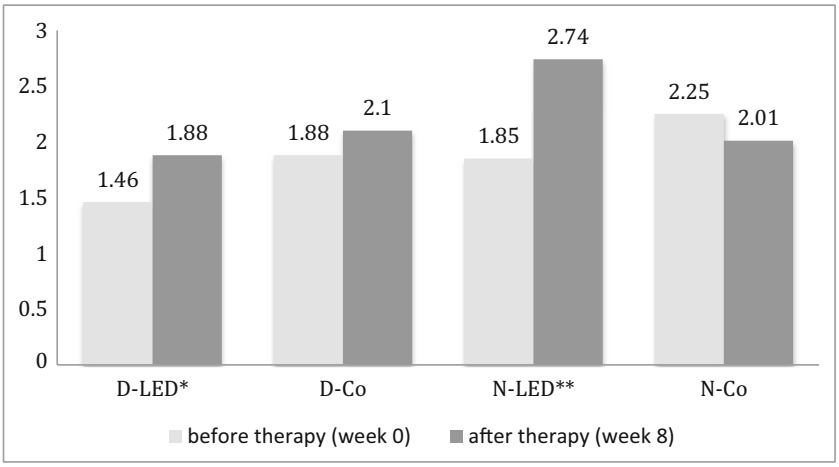

Fig. 3 Mean blood flow measured with LD flux before and after 8 weeks of therapy. In LED-treated groups, a significant increase in blood flow was noted (*$p = 0.040$ and **$p = 0.033$). There was no difference in control groups

cases where a chronic wound is not infected, a surgical approach like skin grafting can successfully be applied.

Another condition that has to be fulfilled in order for a wound to start healing is sufficient blood supply to the wound area. The prognosis of chronic wounds on lower limbs of patients with peripheral angiopathy (not related to the coexistence of diabetes) is directly related to the quality of blood supply to the wound area. Therefore, in cases where healing is impaired due to insufficient blood supply, the possibility of a vascular bypass or endovascular therapy should be considered [29].

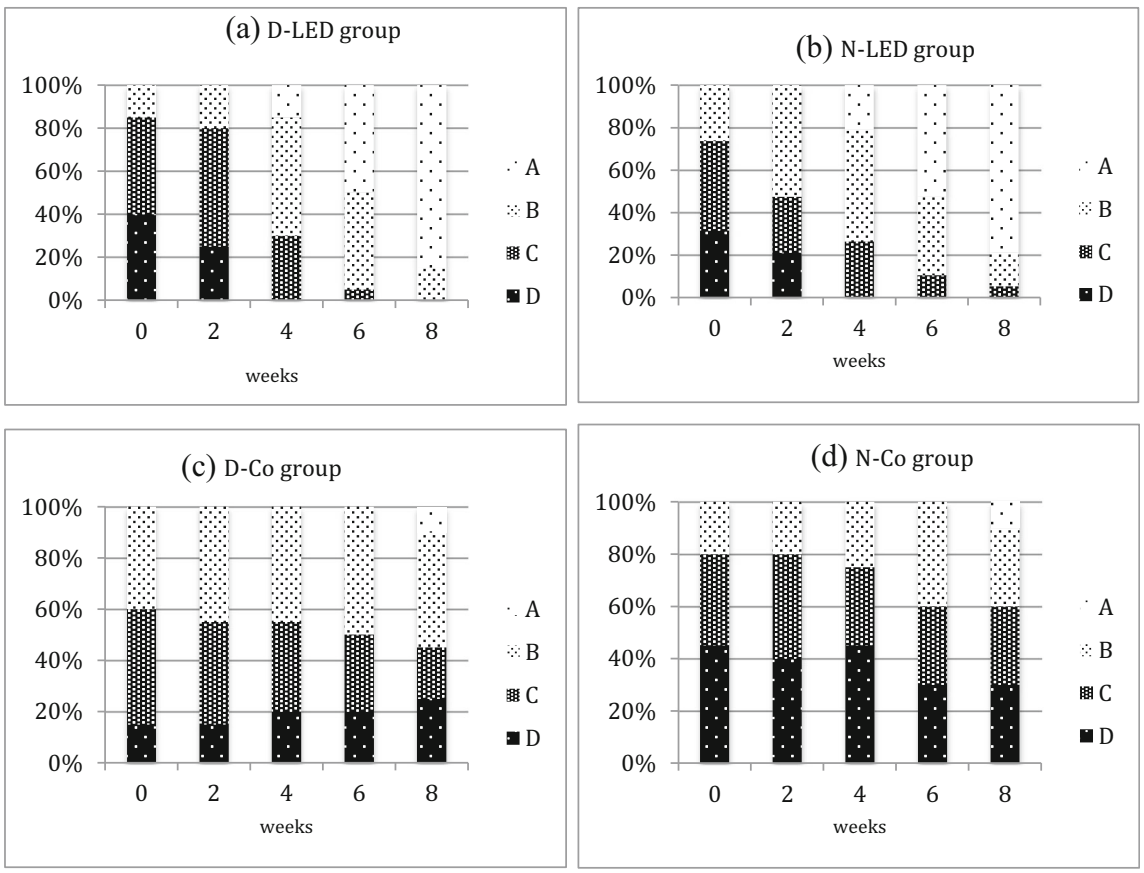

Fig. 4 **a**–**d** The Falanga wound bed score in all groups evaluated every 2 weeks. Before LED therapy (week 0), there was no difference in Falanga score between D-LED and D-Co (p = ns), or between N-LED and N-Co (p = ns). After 8 weeks of treatment, a significant improvement was seen in wound bed granulation (Falanga score A) in both LED-treated groups; D-LED vs. D-Co, $p = 0.0005$; N-LED vs. N-Co, $p = 0.0014$

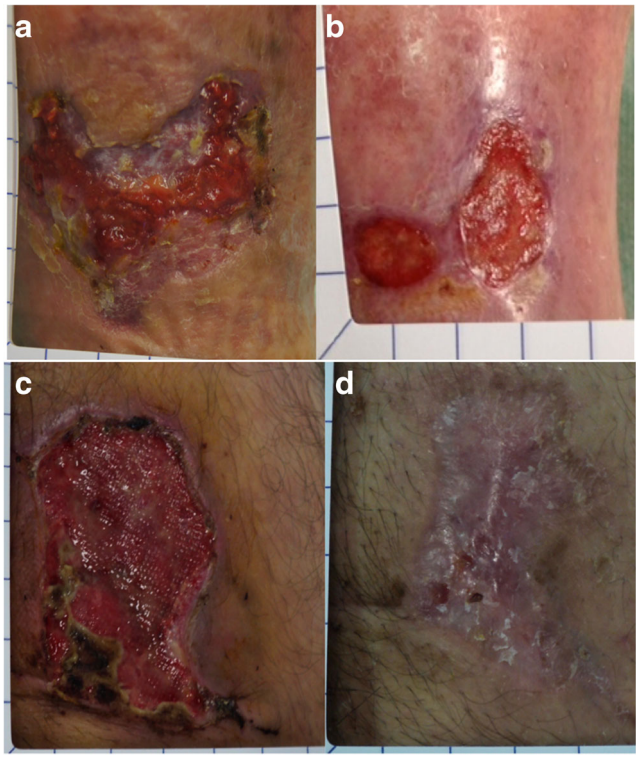

Fig. 5 A 70-year-old female without diabetes that had a persisting wound for 7 months due to peripheral arterial occlusive disease (**a**). The wound was partially healed after 8 weeks of LED therapy (**b**). A 58-year-old male with diabetes and a posttraumatic wound that persisted for 3 months (**c**). After 5 weeks of LED therapy, the wound was completely healed (**d**)

A clinical approach to the treatment of chronic wounds includes different aspects. According to our study results, low-level light therapy with LED has a beneficial effect if used with the right indications.

We evaluated the effect of LED predominantly by measuring the improvement in blood microcirculation using laser Doppler flowmetry. Wound status according to the Falanga wound bed score was also evaluated [27]. Results revealed a significant improvement in wound healing in LED-treated groups according to the Falanga score. Chronic wounds in our research differed in size and depth. Shrinkage of the wound surface during the healing process depends on wound depth; that is why we decided that wound surface would not be the main outcome measure in our study. Falanga wound bed score was also evaluated in our study, but as it is subjective and based on the morphologic appearance of the wound, we looked for a clinically important parameter that could be objectively measured.

Laser Doppler flowmetry (LD flow) provides a non-invasive method for assessing cutaneous perfusion. Skin perfusion measurements using the laser Doppler technique depend on how the light interacts with the moving blood cells and static tissue [30]. In our study, all patients had LD flow measured by the same physician. Measurements were performed before the first LED treatment and after 8 weeks of treatment with LED, on the same area of the intact skin at the wound border.

Study results have shown that microcirculation improved in both groups of patients, diabetics and non-diabetics treated with active LED, as compared to the placebo control groups. The healing process according to the Falanga wound bed score was faster in diabetics and non-diabetics treated with active LED as compared to the control groups.

According to our results, treatment of chronic wounds with LED, if used as an adjuvant therapy to all standard treatment approaches, is effective in diabetic and non-diabetic patients.

Based on previous clinical study results of LLLT in the treatment of diabetic foot ulcers, a beneficial effect was expected. Beckmann reviewed eight randomized clinical trials that all showed an improvement of the wound-healing process according to the main outcome measures that were directly or indirectly associated with wound healing [17]. In our study, the beneficial effect of LED treatment was also seen: the microcirculation and Falanga wound bed status improved after 8 weeks of LED treatment despite the fact that the LED power density used in our study was significantly lower than in most previous studies.

According to Huang and his theory, a negative impact should be expected with higher energy densities used, which is why we decided to use lower doses [31]. But according to Landau, who used 43.2 J/cm^2, and some other reports, healing was importantly enhanced also with much higher energy densities [32–34]. Considering our results and previous study results, the question that arises is as follows: are low energy doses really the most effective or can ineffectiveness in some reports be explained with the use of a dose that was too low? It is known that the total irradiation dose is often impossible to calculate due to a lack of the description of LLLT parameters. Perhaps, wounds of different etiologies require different treatment regimes and leprosy ulcers that did not respond to 2–4 J/cm^2 would exhibit better healing results with higher doses [35]? Or perhaps the reason for a low effect of LLLT in some reports was that only the wound bed and the edges were treated with sources that have a small surface of light beam? In our research, the entire wound area and its surroundings were treated and microcirculation in the healthy skin at wound edge was improved. This means that the blood supply to the wound improved, which is very important because a sufficient blood supply is mandatory for wound healing.

Conclusion

The use of LED as an adjuvant therapy resulted in improved microcirculation and Falanga wound bed score in chronic wound treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Role of funding source There is no funding source.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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