Wound Surface Area and Colony Count of Various Modes of Phototherapy

Zakaria Mowafy Emam Mowafy[1], Ismail Shehata Mostafa Ibrahim[2], Mohamed Bayoumi Ibrahim[1], Amr Mohamed Mohamed Mokhtar Elshahawy[1]
[1]Physical Therapy for Surgery, Faculty of Physical Therapy, Cairo University, Giza, Egypt.
[2]Plastic & Reconstructive Surgery, El- Mataria Teaching Hospital, Cairo University, Giza, Egypt.
*****Corresponding author: Amr Mohamed Mohamed Mokhtar Elshahawy, Mobile: (+20)01156621095,
E-Mail: dr.amr6183@hotmail.com

ABSTRACT
Background: The degree of tissue damage caused by related inflammatory and immunological sequelae poses a major therapeutic treatment problem in burn wounds. Chronic wounds frequently have a significant bioburden and pathogens that are resistant to antibiotics. This review article discusses present research on phototherapy, which is believed to be useful in managing wound bioburden and promoting healing.
Objective: To evaluate the wound healing efficacy of polarized light therapy (BLT) against low-level laser therapy (Ga-As laser) in order to determine which is more efficient and successful at speeding burn healing.
Subject and methods: The current study enrolled thirty patients having partial thickness thermal burn on the forearm (dermal burn). They were selected from Burn Unit at Legislation Association Hospital for Burns & Oncology. They were randomly assigned into two equal groups: Group (A) that included fifteen patients who received the BLT with mean age of 28.8 ± 2.51 years old and group (B) that enrolled fifteen patients who received LLLT with mean age of 29.6 ± 2.79 years old. Both groups also received traditional physical therapy and conservative treatment for the burn site three times weekly for four weeks.
Results: Group A and B showed a significant reduction in colony count and wound surface area after treatment in comparison to that before treatment ($p < 0.001$). In group A, the colony count and wound surface area decreased by 38.63% and 55.1% respectively, but in group B, by 15.6% and 37.92% respectively.
Conclusion: Both polarized light therapy and low-level laser therapy had a therapeutic efficiency on wound healing, but BLT is more efficient and more successful in the acceleration of the burned wounds healing.
Keywords: Burn, Wound healing, Colony count, Low-level laser, Polarized light therapy.

INTRODUCTION

Burns are typically described as skin wounds due to thermal exposure (e.g., fire, hot liquids, solids, or gases), electricity, radiation exposure (e.g., ultraviolet light, ionizing radiations such as X-ray, microwaves, etc.), or chemical compounds (e.g., strong acids or bases). The main treatment objective is prevention and management of infection while promoting adequate healing and function preservation [(1)]. Patients who have sustained severe burns covering a large part of their body surface require rapid skilled emergency treatment to reduce death and frequently require long-term rehabilitation and recovery to avoid disabling scars and other morbidities [(2)]. Certain conventional therapies depend on the use of topical antibiotics, mafenide acetate, chlorhexidine, povidone-iodine, or silver-sulfadiazine [(3)]. Recently, light therapy, such as low-level laser therapy as a coherent source of light and polarized polychromatic noncoherent light (Bioptron) therapy as a polychromatic and non-coherent light source, has been suggested as a non-aggressive treatment choice in case of slow-to-heal or non-healing wounds in soft tissues, tendons, and bones, owing to enhanced tissue nutrition and oxygenation [(4)]. Bioptron light treatment promotes endogenous purification through cellular debris and infectious pathogens removal by: boosting macrophage stimulation, improving bacterial phagocytosis activity and capacity through increased scavenger cell creation, increasing neutrophil stimulation, increasing neutrophil number and phagocytosis activity [(5)]. Low-level laser therapy (LLLT) is progressively being utilized to minimize complications of burn because it promotes tissue regeneration and wound healing, while also reducing pain and edema via anti-inflammatory mechanisms that increase adenosine triphosphate (ATP) synthesis, decrease oxidative stress and stimulate natural biological processes [(6)]. Additionally, numerous in vitro investigations have demonstrated that LLLT stimulates human gingival fibroblasts proliferation and decreases inflammatory conditions [(7)].

The objective of the present study was to evaluate the wound healing efficacy of polarized light therapy (BLT) against low-level laser therapy (Ga-As laser) in order to determine which is more efficient and successful at speeding burn healing.

PATIENTS AND METHODS

The study enrolled thirty patients who had partial thickness thermal burn on the forearm (dermal burn). They were selected from Burn Unit, Legislation Association Hospital for Burns & Oncology. They ranged in age from 25 to 35 years and were randomly assigned into two equal groups:

Group (A): Included 15 male patients who underwent BLT plus traditional physical therapy routine and

Received: 23 /6 /2021
Accepted: 19 /8 /2021

conservative treatment for the burn wound three times /week for four weeks as a total period of treatment.

Group (B): Included 15 male patients who underwent LLLT plus traditional physical therapy routine and conservative treatment for the burn wound three times /week for four weeks as a total period of treatment.

Ethical approval:

Research Ethics Committee and quality control approvals were obtained. Each participant was informed about the research objectives and methods in detail and using simple language prior to being requested to provide written informed consent prior to participation in the research. The trial coordinator performed regular quality control of screening, data management, and protocol adherence. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion Criteria:

1- Their average age was 25-35 years old.
2- All patients had dermal burns involving one of the forearms with TBSA of (15-25%).
3- The cause of burn for all patients was thermal injuries.
4- All patients were conscious and can perform the protocol of evaluation tests and treatment properly.
5- Every patient signed a consent form.

Exclusive Criteria:

1- Patients suffering from diabetes.
2- Patients suffering from skin abnormalities (skin malignancy in the area to be treated).
3- Patients suffering from anemia.
4- Patients suffering from associated or inhalation injuries.
5- Post-skin grafting patient.
6- Patient with life-threatening disorders as renal failure or myocardial infarction.
7- Patients who are pregnant.
8- Patients suffering from hemorrhage, acute tuberculosis, myasthenia gravis, hyperthyroidism, mental disorders, acute viral disease, or those with pacemakers.

Procedures of the study:

The procedure of the study was divided into two categories:

1-Measurement procedures:

The evaluation was conducted before and after the treatment.

a. Wound surface area: The wound surface (WAS) was determined using the planimeter method by putting a sterilized transparency film over the forearm wound and marking the wound perimeter on the film with a fine-tipped transparency marker. Each wound will be represented by distinct transparency. After that, the tracing is placed on metric graph paper, and the number of 1mm marks on the tracing is counted (only full 1 millimeter squares inside the perimeter are counted, and the area is converted to square centimeters). The wound area was measured before the beginning of the experiment and after the second month of treatment ends [8].

b. Colony count:

Semi-quantitative culture of the wound surface area

1- A sterile cotton swab has been fully rolled over the forearm wound site.
2- The swab material was thoroughly emulsified in a sterile 5 ml (0.9% NaCl).
3- Three serial 1:10 dilutions of the suspension using 0.5 ml aliquots and 4.5 ml sterile saline per aliquot were performed.
4- A 0.1 ml sample of the initial solution and of each dilution was distributed over the blood gar plate surface.
5- All plates were incubated for 24 hours at 37°C.
6- The number of organisms per milliliter of the swab solution is measured by counting the colonies that developed between 30 and 300 colonies on the plate.
7- To determine the number of colonies on the plate, multiply the count in step 6 by the dilution factor.
8- Each colony was counted separately.
9- To undertake a preliminary identification of each colony count, the Gram stain and critical tests such as oxidase and catalase assays, as well as colony morphology, has been done [9].

2-Therapeutic procedures:

Therapeutic procedures:

All patients in the studied groups got identical traditional physical therapy routine and conservative wound management, same nursing care, same medications and described diet. Dressing was the same for all patients in both groups where it was covered by sterile vaseline gauze and changed once daily [10].

Steps of the BLT treatment procedures in the first group (A):

1. Patient positioning: For the upper limb burns, the supine lying position was excellent.
2. Wound preparation: The wound was cleaned at first by hydrogen peroxide, saline rinse and betadine.
3. BLT device preparation: The BLT unit's plug is plugged into the main power supply, and the on/off switch is turned on. Then adjust the BLT treatment settings.
4. BLT application: Direct the laser beam toward the areas needing treatment for about 10 minutes, with the device held at a right angle (90°) and at a 10 cm distance from the burn wound's surface.
5. Application frequency: One time per day, 3 times weekly for one month or until healing occurs [11].
6. Unplug the device after usage, and consider extending the BLT by one or two weeks if the

wound closes before the course of treatment (one month) ends for strengthening the treated region.

Procedures of LLLT for the second experimental group (B):

For one month, both BLT and LLLT has been administered one time per day, three times weekly or until wound healing.

In this study, the treatment protocol presented under the following:

- Patients were informed about the measurement and therapeutic methods, and also the BLT, laser device, and laser irradiation prior to the starting of treatment.
- Patients were instructed to adhere to the surgeon's and physical therapist's instructions.
- Patients were asked to avoid predisposing factors like UV rays, crowding and unclean places, hot and humid environments as well as smoking.
- Measurement procedures were applied for each patient, as they were mentioned in the measurement section.
- Before therapy, all patients were given their written informed consent form for the BLT and laser treatment.
- The eyes were protected from BLT or laser irradiation.
- Assemble a comfortable posture for the patient.
- Before starting treatment, ensure that the device is turned off.
- Select the appropriate dose.
- Switch on the device.
- After the treatment ends, switch the device off and then cheek the treated area.
- Ga-As laser with an 850 nm wavelength, a 200 mV power output in pulsed mode, continuous-wave, and a 0.07 cm^2 spot area was used in the study.
- The laser source probe was in light contact with the burn surface at the center of each square and was aimed at a 90-degree angle to the injured tissue during the assigned treatment time period. Dosages of 1.2 J/cm^2 and 2.4 J/cm^2 at a maximum of 6 points for 1 minute per point were used in the burn regions .The laser device was applied within the time limit, and dosage at every centimeter along the burn wound edges in non-contact mode, five times a week. Protective eyeglasses were worn by the physician and patient during the laser therapy sessions to ensure safety [12].

Statistical analysis

To compare age and TBSA among groups, descriptive statistics and an unpaired t-test were used. The chi-squared test was used to compare the gender distribution of the groups. The Shapiro-Wilk test was used to determine the normal distribution of data for all variables. To determine the homogeneity of variances between groups, Levene's test was used. To compare the mean values of wound surface area and colony count between groups A and B, unpaired t-test was used. A paired t-test was used to compare each group before and after treatment characteristics. All statistical tests were conducted with a significance level of $p \leq 0.05$. All statistical analyses were performed using IBM SPSS version 25 for Windows (Chicago, IL, USA).

RESULTS

Subject characteristics:

Table (1) summarizedthe characteristics of the subjects in the studied groups. The mean age, TBSA, and gender distributions did not differ significantly among the two groups (p > 0.05).

Table (1): Comparison of subject characteristics among the studied groups

	$\bar{x}\pm$ SD		p-value
	Group A	**Group B**	
Age (years)	28.8 ± 2.51	29.6 ± 2.79	0.41
TBSA (%)	19.46 ± 2.5	19.86 ± 2.92	0.69
Sex			
Females	2 (13%)	3 (20%)	0.62
Males	13 (8%7)	12 (80%)	

P-value, probability value; SD, standard deviation; $\bar{x}$, mean

Treatment effect on wound surface area and colony count:

Comparison within-group: Among the studied groups, colony count and wound surface area showed a significant reduction post-treatment compared to pre-treatment (p < 0.001). The colony count and wound surface area decreased by 38.63% and 55.1% respectively in group A, but in group B, by 15.6% and 37.92% respectively (Table 2).

Comparison between groups: As shown in table (2), pre-treatment colony count and wound surface area did not differ significantly among groups (p > 0.05). After treatment, a significant reduction in colony count and wound surface area was detected in group A compared to group B (p > 0.001)

Table (2): Mean wound surface area and colony count before and after treatment among the studied groups

	Group A	Group B			
	x̄± SD	x̄± SD	MD	t- value	p value
Wound surface area (cm^2)					
Pre treatment	6.17 ± 1.55	6.33 ± 1.2	-0.16	-0.31	0.75
Post treatment	2.77 ± 0.9	3.93 ± 0.74	-1.16	-3.82	0.001*
MD	3.4	2.4			
%of change	55.1	37.92			
t- value	13.69	11.16			
	p = 0.001*	**p = 0.001***			
Colony count ($x\ 10^{10}$)					
Pre treatment	13.46 ± 2.85	13.66 ± 2.61	-0.2	-0.2	0.84
Post treatment	8.26 ± 2.37	11.53 ± 2.5	-3.27	-3.66	0.001*
MD	5.2	2.13			
% of change	38.63	15.6			
t- value	12.49	12.91			
	p = 0.001*	**p = 0.001***			

p-value, probability value; SD, standard deviation; x̄, mean; MD, mean difference; *, significant

DISCUSSION

The present study aimed to assess the therapeutic efficiency of polarized light therapy (BLT) versus low-level laser therapy (Ga-As laser) on wound healing and detect which one will be more efficient and successful at speeding burn healing.

The current study enrolled thirty patients, having partial thickness thermal burn on the forearm (dermal burn). Their ages ranged from 25 to 35 years old. They were assigned into two equal groups in a random fashion: Group (A) that included fifteen patients who received the BLT and group (B) included fifteen patients who underwent LLLT. Both groups also received traditional physical therapy and conservative treatment for the burn site three times a week for four weeks.

The wound surface area and colony count data from the studied groups were statistically analyzed and compared.

Among the studied groups, wound surface area and colony count showed a significant reduction after treatment compared to that before treatment ($p < 0.001$). The wound surface area and colony count decreased by 55.1% and 38.63% respectively in group A, but by 37.92% and 15.6% respectively in group B. Pre-treatment wound surface area and colony count did not differ significantly among groups ($p > 0.05$). After treatment, comparisons among groups showed a significant decrease in wound surface area and colony count in group A in comparison with group B ($p > 0.001$).

As a consequence of the preceding findings, it is apparent that the improvement in burn wound healing among the studied groups is related to a significant reduction in wound surface area and colony count. This greater enhancement in chronic wound healing in group A than in group B may be explained by the fact that BLT has bio-stimulatory effects. On skin application, it activates light-sensitive intracellular biomolecules. This starts chain reactions at the cellular level and also induces secondary responses that are not restricted to the treated skin region but can have an effect on the entire body [(13)].

Bio-positive effects attributed to Bioptron include increasing anti-inflammatory cytokine levels and fibroblast proliferating factors, reducing plasma levels of pro-inflammatory cytokines, and modifying lymphocyte proliferation [(13)]. Polarized light also boosted the body self-defense systems, and microscopic studies demonstrated a significant rise in the number of white blood cells such as eosinophils, monocytes, and lymphocytes after polarized light therapy [(14)]. Additionally, it was demonstrated that polarized light increased T-lymphocyte activity in the blood. Furthermore, polarized light was shown to stabilize the erythrocyte cell membrane, which binds more oxygen molecules and transports them to all organs and cells in the body [(15)]. Our findings are in agreement with **Medina and Lens** [(16)] who evaluated the efficacy of polarized, polychromatic, non-coherent light therapy in treating venous leg ulcers. Over a four-week period, phototherapy was administered once a day. Except for one ulcer, all of the 73 ulcers had a positive value due to the increased healing rate and decreased wound surface area. Following treatment, there was a statistically significant reduction in wound surface area (mean 57.15 %, SD: 31.87 %, and P-value < 0.01). They concluded that polarized, polychromatic light therapy was associated with positive healing rates. Additionally, **Durovic *et al.*** [(17)] reported that after four weeks of polarized light therapy, a significant improvement was detected in twenty patients having stage I-III ulcers as regards their pressure ulcer healing, indicating that polarized light may be beneficial in treating pressure ulcers. After the completion of therapy, significant differences existed among groups. The experimental group improved significantly compared to the control group in terms of total PUSH score, pressure ulcer rank, and pressure ulcer surface area. Moreover, **Iordanou *et al.*** [(18)] investigated the impact of Bioptron treatment in addition to conventional care on 55 in-patients having pressure ulcers. After the completion of weeks one and two, statistically significant differences between treated and untreated ulcers were detected. The treated group's ulcers decreased in size by a mean of 10.56 % in size, compared to 0.95 % in the control group. These findings support those of **Monstrey *et al.*** [(5)] who reported that daily Bioptron polarized therapy hastened wound closure in twenty-two patients having severe second-degree burns, significantly reduced healing time, decreased scarring, and improved long-term functional outcomes.

Furthermore, **Simic *et al.*** [(19)] assessed Bioptron treatment versus conventional care in twenty-six patients who underwent complete gastrectomy and were left having left thoracophrenolaparotomy incisions measuring about 42 cm across the chest and abdomen. On the 12th postoperative day, results were significantly better in the Bioptron group.

The current study's findings are consistent with those of **Pinheiro *et al.*** [(20)] who found that using 685 nm polarized light at a dosage of 20 J/cm^2 enhanced collagen deposition and wound healing, as well as increasing the number of myofibroblasts. **Trelles *et al.*** [(21)] and **Watson *et al.*** [(22)] also demonstrated that fibroblasts at the injured dermis edge might be converted into myofibroblasts, whose contractile nature and smooth muscle actin fibers can limit wounds progression. This may be consistent with the present study findings, which revealed a greater enhancement in wound surface area during the first week, indicating a greater response to PLT.

The study was confined to patients' physical and psychological problems that might influence their assessment and treatment.

CONCLUSION

It may be stated that after four weeks of therapy, patients in both groups demonstrated progress in their burn healing. Both polarized light therapy and low-level laser therapy have been shown to be beneficial in the treatment of a variety of wounds and wound healing problems. They have the potential to enhance and accelerate wound healing by stimulating and modulating regeneration processes, inhibiting inflammation, and boosting the human defense system activities. However, the polarized light treatment appears more efficient than low-level laser therapy in speeding the process of recovery and reducing hospital stay duration.

Future studies and Recommendations:

The results of this study indicated a need to consider the following recommendations:

1. It was recommended to add phototherapy as an integral part of treatment for burned patients.
2. Conducting more similar studies using other types of phototherapy modalities and for a longer time duration, as well as increasing the patients' number in addition to comparing them with the results of this study to obtain better statistical results to reach the best method of treatment.
3. Additional research is necessary to assess the phototherapy effect on physical performance and quality of life for patients after burn.

Financial support and sponsorship: Nil.

Conflict of interest: Nil.

REFERENCES

1. **Peck M, Molnar J, Swart D (2009):** A global plan for burn prevention and care. Bulletin of the World Health Organization, 87: 802–803.
2. **Dai T, Huang Y, Sharma S *et al.* (2010):** Topical antimicrobials for burn wound infections. Recent Patents on Anti-infective Drug Discovery, 5: 124–151.
3. **Sevgi M, Toklu A, Vecchio D *et al.* (2013):** Topical antimicrobials for burn infections - an update. Recent Patents on Anti-infective Drug Discovery, 8: 161–197.
4. **Yin R, Dai T, Avci P *et al.* (2013):** Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. Curr Opin Pharmacol., 13: 10-15.
5. **Monstrey S, Hoeksema H, Depuydt K *et al.* (2002):** The effect of polarized light on wound healing. European Journal of Plastic Surgery, 24 (8): 377- 82.
6. **Boschi E, Leite C, Saciura V *et al.* (2008):** Anti-Inflammatory effects of low-level laser therapy (660 nm) in the early phase in carrageenan-induced pleurisy in rat. Lasers Surg Med., 40: 500–508.
7. **Almeida-Lopes L, Rigau J, Zângaro R *et al.* (2011):** Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. Lasers Surg Med., 29: 179–184.
8. **Kloth L (2005):** Electrical stimulation for wound healing: A review of evidence from vitro studies, animal experiments and clinical trials. Int J Low Extrem Wounds, 4 (1): 23-44.
9. **Barrick B, Campbell E (1999):** Leukocyte Proteinases in Wound Healing: Roles in Physiologic and Pathological Processes. Wound Repair Regen., 7: 410-422.
10. **Wayne P (2001):** Healing and Chronic Wound Management" 3rd ed. Health Management Publications. Pp: 7-17.

11. **Medenica L, Lens M (2003):** Pressure ulcers and the polarized light therapy. J wound Care, 12: 37-40.
12. **Eun J, Sehun K (2017):** Anti-inflammatory effects of low-level laser in burn wound models in rats. Physical Therapy Rehabilitation Science, 6: 170-5.
13. **Reddy M, Gill S, Kalkar S *et al.* (2008):** Treatment of pressure ulcers: a systematic review. JAMA., 300: 2647–62.
14. **Begic-Rahic J, Vranic S (2010):** The application of bioptron light therapy in dermatology and wound healing. European Dermatolo., 5: 57–60.
15. **Fenyo M (1984):** Theoretical and experimental basis of biostimulation. Optics & Laser Technology, 16: 209–215.
16. **Fenyo M, Mandl J, Falus A (2002):** Opposite effect of linearly polarized light on biosynthesis of interleukin-6 in a human B lymphoid cell line and peripheral human monocytes. Cell Biol Int., 26: 265–269
17. **Durovic A, Maric D, Brdareski Z *et al.* (2008):** The effects of polarized light therapy in pressure ulcer healing. Vojnosanitetski Pregled., 65 (12): 906-12.
18. **Iordanou P, Lykoudis E, Athanasiou A *et al.* (2009):** Effect of visible and infrared polarized light on the healing process of full-thickness skin wounds: An experimental study. Photomedicine and Laser Surgery, 27 (2): 261-7.
19. **Simic A, Pesco P, Bjelovic M *et al.* (2001):** Bioptron light therapy and thoracophrenolaparotomy wound healing in patients operated due to cardiac carcinoma, paper presented at the 4th International Gastric Cancer Congress, New York, USA. https://apgaylard.wordpress.com/2009/02/28/cancer-and-the-magic-lamp/
20. **Pinheiro A, Pozza D, De Oliveira M (2005):** Polarized light (400–2000 nm) and non-ablative laser (685 nm): A description of the wound healing process using immunohistochemical analysis. J Photomed. Laser Surg., 23: 485–492.
21. **Trelles M, Allones I, Mayo E (2006):** Combined visible light and infrared light emitting diode (LED) therapy enhances wound healing after laser ablative resufasing of photodamaged facial skin. Med Laser Appl., 21: 165–175.
22. **Watson M, Wallace K, Gieling R *et al.* (2008):** NFkappaB is a critical regulator of the survival of rodent and human hepatic myofibroblasts. J Hepatol., 48: 589–97.