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808-Nm Near-Infrared Laser Photobiomodulation versus Switched-Off Laser Placebo in Major Aphthae Management: A Randomized Double-Blind Controlled Trial

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Featured Application: An effective and repeatable photobiomodulation therapy can contribute to increasing pain relief, enhancing healing and tissue repair processes. Our data can support the development of more commercial products for easier home use in the future.

Abstract: Background: the aphtha is one of the most common oral mucosal ulcerations and presents as a painful punched-out sore. Systemic and topical medications are used to reduce inflammation and pain and to support the natural period of remission. Alternative treatment modalities have been requested to relieve pain and improve its healing. In this regard, photobiomodulation, which is a manipulation of cells' metabolism through an energy transfer by light sources of non-ablative or thermal intensity, could support aphtha management. The predictor variable of our research was the photobiomodulation through higher energy and power irradiated through a handpiece with a flat-top beam profile. The primary end point was the complete healing of the aphtha, defined as the time from the irradiation to the complete recovery. The secondary end point was pain relief, evaluated daily through the visual analogue scale (VAS), from the irradiation to 24 and 48 h after. Methods: a randomized, double-blind, controlled trial was conducted according to the CONSORT guideline. Irradiation was performed through an 808-nm diode laser with flat-top handpiece, and 1 W, 1 W/cm^2, 60 J, 60 J/cm^2 for 60 s on a spot-size area of 1 cm^2. Time of complete healing and pain evaluation by VAS scale were evaluated. Results: between 1 January 2020 and 1 March 2021, 126 patients were screened for the study at the Department of Surgical and Diagnostic Sciences, University of Genoa, Italy. Sixty patients were randomly assigned (30 in the photobiomodulation group and 30 in the placebo group). Patients of the photobiomodulation group experienced complete healing in an average time of 8.13 days $\pm$ 1.69 (min 5–max 10 days), while for the placebo group the average time extended to 30.76 $\pm$ 4.63 days (min 25–max 42 days). Patients of the photobiomodulation therapy group experienced a statistically significant reduction in pain and discomfort 24 and 48 h after treatment ($p < 0.05$); the reduction was statistically higher ($p < 0.05$) 48 h after treatment compared to 24 h after. Conclusions: photobiomodulation at the parameters and modality of irradiation proposed accelerates the healing recovery and reduces pain compared to the patients treated with the placebo.

Keywords: aphthous ulcers; mouth ulcers; canker sores; ulcer healing; aphthous stomatitis; oral ulceration; oral mucositis

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1. Introduction

Due to the anatomical characteristics, high vascularity, and the peculiar functions of the oral cavity, the oral mucosa is vulnerable to many insults. These include systemic diseases and the effects of autoimmune disorders, trauma from oral appliances or chewing, and local skin rashes, following exposure to systemic drug therapies, ingestion of irritating food or drink, and infectious strains [1]. Adverse stimuli can produce oral manifestations ranging from lichenoid lesions to hyperkeratosis and ulceration. Aphthous stomatitis (AS) is an ulcer disease that commonly affects the oral mucosa. It presents as painful ulcers with shallow erythematous margins and a yellowish-grey pseudomembranous center [2]. The percentage in the world of adults affected by AS in its recurrent manifestation [(recurrent aphthous stomatitis (RAS)] is about 20%, with variations from 5% to 50%, while in children the prevalence is 39% [2,3]. AS comes in three forms, which are morphologically classified as minor aphtha or Mikulicz-ulcers, which show a diameter of 2–10 mm, major aphtha, also called mucosal necrotic periadenitis or Sutton ulcers (>10 mm in diameter), and herpetiform ulcerations, which consist of multiple small ulcers [4]. No causal treatment for RAS has been developed so far; systemic and topical medications are used to reduce inflammation and pain and to support the natural period of remission [5]. Management of oral ulcers is based on the severity of the lesions and usually involves the administration of corticosteroids [5]. However, there is a lack of high-efficacy evidence for them [6], particularly for systemic interventions. The most used protocol is, therefore, a combination of topical systemic agents (colchicine, pentoxifylline, corticosteroids, dapsone, thalidomide, cyclosporine, and infliximab), a vestibular antiseptic or anti-inflammatory therapies (chlorhexidine gluconate, triclosan, amlexanox), and local anesthetics (benzocaine gel), as well as local antibiotics (tetracycline, chlortetracycline) and topical steroids (triamcinolone acetonide). Furthermore, trigger foods and acidic foods and drinks should be avoided [1,4,7]. These primarily symptomatic therapies may, however, induce side effects; steroid medications can cause candidiasis, and tetracycline or chlorhexidine mouthwashes can shorten ulcer duration but are contraindicated in children and may induce tooth discoloration [5]. Therefore, alternative treatment modalities have been developed to relieve pain and improve AS healing, such as traditional Chinese medicine [8], acupuncture [9], psychotherapy [10], and low-level/intensity laser therapy (LLLT) [11,12]. LLLT, now more correctly reported as photobiomodulation, can be considered "a manipulation of cells' metabolism through an energy transfer by light sources of non-ablative or thermal intensity" [12]. At the cellular level, the features of photoreceptors allow animals and humans to experience the ability to respond to light stimuli and influence the visual processes and vitamin D production. However, ubiquitous and unspecialized molecules called photoacceptors are "in the spotlight" because of their energetical role in the cell-light interaction [13,14]. Photons, as bosons, can transfer their energy to photoacceptors, inducing a photochemical reaction in the cell [15]. As we recently described [16], photobiomodulation is based on biological and physical–chemical evidence, where the pivotal player is the mitochondrion, whether its cytochromes are directly involved as a photoacceptor or indirectly through a vibrational and energetic variation of bound water and lipids.

Indeed, from a cellular point of view, the primary interaction with the photoacceptors, such as the cytochromes of the mitochondrial respiratory chain, the lipids, the nitrosothiol compounds, and the bounded water and the modification of their energetic and vibrational state, supports the release of adenosine triphosphate (ATP), reactive oxygen species (ROS), nitric oxide, as well as calcium, which is liberated through voltage-dependent receptors or released from sequestered reserves of the organelles [16,17]. From a medical point of view, the energized cells and the activation of cellular pathways can resolve in improving inflammation, regeneration, and more generally, in the homeostasis recovery of metabolic dysfunction of the tissues [18]. Concerning the effect of photobiomodulation on AS, in our recent review [12], we concluded that "photobiomodulation is a promising treatment modality. However, due to the heterogeneity of the literature's data, photobiomodulation needs further testing through well-designed, long-term, and randomized controlled trial

studies, allowing to evaluate it with diligent and impartial outcomes". For instance, our screening of literature according to the PRISMA guideline through PubMed/MEDLINE and Google Scholar databases, using the keywords—Low-Level Laser Therapy OR Photobiomodulation and aphtha OR aphthae OR aphthous (inclusion criteria: indexed papers written in the English language, peer review process, clinical studies, full research articles, description of therapy parameters following the criteria suggested by Tunér and Jenkins [19]), found more than 900 papers, which was reduced to 76 and then 12 eligible works [20–31]; however, the articles were omitted if both the evaluation of irradiated energy by a power meter and the temperature measure before and after irradiation were requested for eligibility. Plus, many therapies were administered, starting from a non-indicated distance from the lesion with the operator slowly approaching the lesion, or in other cases, the distance from the affected area was kept "quite constant", but the fiber or handpiece was moved in a circular or "painting-like" fashion [21,25]. Besides, the irradiation time indicated a lack of accuracy and it is described as being of only a "few seconds", or the therapy is administered with intervals of a few, but not a precise number of minutes between the two or more repeated irradiations; the actual irradiated parameter can therefore change from one operator to another, influencing the photobiomodulation therapy reliability and repeatability.

To improve the clinical knowledge and the support of photobiomodulation in the treatment of aphtha healing and the standardization of the therapies, we thus set up a randomized double-blind controlled trial. In our previous works, we showed that a higher-power and -fluence laser therapy, such as 808-nm, 1 W, 1 W/cm^2, 60 J/cm^2, positively affects mitochondrial energetic metabolism [32–34], improving healing [35–38] in in vitro and preclinical studies. Plus, we characterized a novel technology based on irradiation with a flat-top beam profile that can be supportive of the effectiveness and standardization of photobiomodulation therapy [32,39] and Section 2.3 below.

Therefore, the predictor variable of our research was the photobiomodulation through higher energy and power irradiated through the flat-top handpiece. The primary end point was the complete healing of the aphtha, defined as the time from the irradiation to the complete recovery. The secondary end point was pain relief, evaluated daily through the visual analogue scale (VAS), from the irradiation to 24 and 48 h after.

2. Materials and Methods

2.1. Study Design and Participants

Our randomized double-blind placebo-controlled trial was experimentally performed at the Department of Surgical and Diagnostic Sciences (DISC), University of Genoa, (Genoa, Italy). Eligible patients were females or males affected by major aphtha, which occurred no more than 48 h before the visit. The aphtha had to be located in three main oral districts, such as fornixes, the floor of the oral cavity, and the jugal mucosa. The degree of erythema and exudation were evaluated by the investigators on a 4-point scale (range 0–3) based on the methods of Greer et al. [40].

Patients were excluded if they showed diabetes, autoimmune diseases, HIV, HBV, HCV, tumor (for example, an ulcerated squamous cell carcinoma) or were smokers, pregnant women, or younger than 18 years. Additionally, patients were also excluded if they had herpetic lesions or systemic disease that predisposed them to have RAS.

The trial protocol was approved by the Regional Ethical Review Board of DISC, the University of Genoa (Unige-DISC-protocol number 0015098). The study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and applicable local laws. All patients provided written informed consent. The trial complied with the CONSORT guideline.

2.2. Randomization and Masking

Patients were randomly allocated (1:1) to either photobiomodulation or placebo therapies. An anonymous numbered questionnaire was used to gather patients' screening information. Randomization was based on a sequentially numbered, opaque, sealed envelope (SNOSE) technique to assign the treatment. The placebo therapy was applied with the laser for the photobiomodulation therapy but with the device switched off. A 635-nm red-light guide (negligible power) with the laser device switched to silent mode was used to keep the irradiation of the therapies blinded. Patients and study team members responsible for the administration of the treatment were therefore were masked toto treatment allocation. Two experienced clinicians other than the operators involved in irradiation therapies, which were calibrated to 100% intra-examiner agreement for all the evaluated criteria before the study, performed an independent assessment of the healing during the evaluation time. The two examiners were blinded to the two experimental groups. Investigators responsible for data analysis were also masked to treatment assignment.

2.3. Procedures

To improve the standardization and the repeatability of the photobiomodulation therapy, the 808-nm diode laser device (Doctor Smile, LAMBDA Spa, Vicenza, Italy) was used with a novel handpiece able to irradiate through a flat-top beam profile (AB 2799, Doctor Smile, LAMBDA Spa, Vicenza, Italy). Indeed, authors [39,41] explain that the divergence of the beam, the beam profile, and the diameter of the final emission device strongly influence the positive effect of the photobiomodulation. Firstly, the applied energy is distributed over an increasing area as the tip-to-tissue distance increases, greatly affecting the energy density at the cellular level [39]; in some cases, the energy density can be reduced by 95% with only 5 mm of target distance [41]. Secondly, if the use of a collimated beam with a predetermined diameter could resolve the problem, the laser energy emitted by an optical fiber or a therapeutic handpiece has a Gaussian profile; it is not uniformly distributed on its emission surface, and it therefore has a higher photon density at the center of the ray than at the edge [32,39,41].

In accordance with our previous in vitro and preclinical studies [35–38], photobiomodulation therapy was administered through the power of 1 W irradiated in continuous wave mode (CW) for an exposure time of 60 s and on a spot size of 1 cm^2, which allowed generating a power density of 1 W/cm^2 and a fluence of 60 J/cm^2 (energy administered = 60 J). Because the aphtha's area is wider than the laser spot size, irradiation on two points was necessary to cover the entire surface. The placebo therapy was processed under identical conditions, except the laser device was kept off at all times (0 W).

The precision of the laser therapy parameter was secured by the Pronto-250 power meter (Gentec Electro-Optics, Inc., Quebec, QC, Canada).

Adverse events due to a possible undesirable thermal effect were avoided by monitoring the irradiation with a thermal camera FLIR ONE Pro-iOS (FLIR Systems, Inc., designs, Portland, OR, USA) (dynamic range: −20 °C/+400 °C; resolution 0.1 °C).

All randomized patients, including those assigned to the placebo, underwent an evaluation of the aphtha area, pain, and discomfort.

The area was measured by NIH image analyzer (National Institute of Health, Rockville, MD, USA). Basically, images of the aphtha and the periodontal probe, which was used to set the measure through the NIH image program, was obtained by Canon EOS 450 camera, lens of 100 mm (Canon Italia S.p.A, Cernusco Sul Naviglio, Milan, Italy). The images were acquired in a standard condition of illumination, exposition, distance, and tilt angle.

The pain was evaluated by the visual analogue scale (VAS) (Figure 1), asking the patients to draw a line along a 10-cm horizontal that best describes their pain, with the extremes of the lines representing no pain and the worst pain possible. The VAS was then scored by measuring the distance between the patient's line and the no-pain anchor.

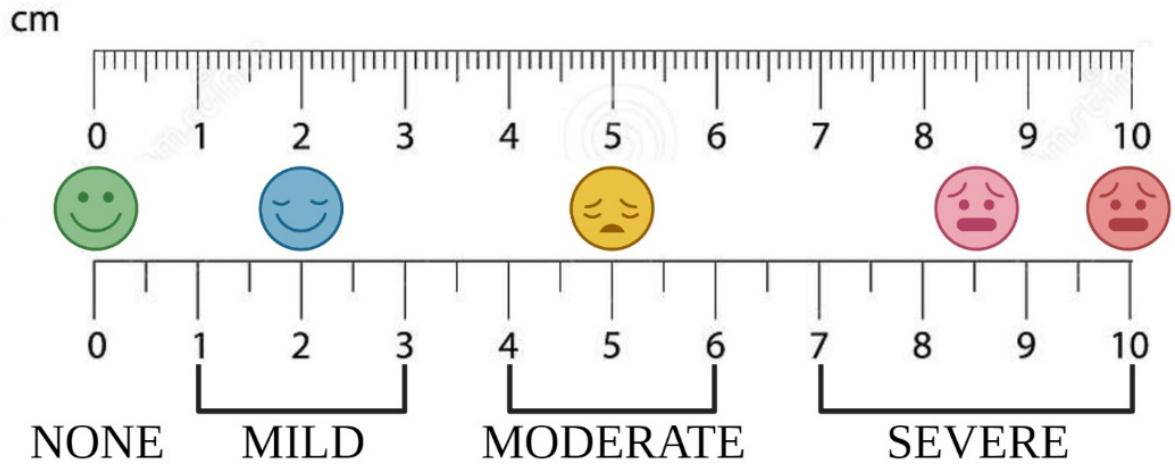

Figure 1. Visual analogue scale (VAS) evaluating the pain induced by aphtha. Image created with BioRender.com. (accessed on 1 April 2021).

Evaluations were performed immediately before the irradiation and 24 and 48 h after.

Patients based their evaluation on compliance with the following questions: what was your subjective experience of eating today? What was your subjective experience of drinking today? What was your subjective experience of brushing your teeth today?

2.4. Outcomes

In accordance with our previous works [35–38], the predictor variable was the photobiomodulation through higher energy and power irradiated through the flat-top handpiece.

The primary end point was the complete healing of the aphtha, defined as the time from the irradiation to the complete recovery.

The secondary end point was pain relief, evaluated daily through the VAS scale, from the irradiation to 24 and 48 h after it.

2.5. Statistical Analysis

Primary and secondary outcome measures were statistically analyzed. The mean ± standard deviation of measures and score data, as well as the statistical power of the sample size, were taken into account. In the assessment of the data, a linear mixed model was used. Calculations were performed using the SPPS 25 (IBM Corp. Released in 2017. IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY, USA) statistics package program. For the significance level of the tests, the value of $p < 0.05$ was accepted.

The sample size was calculated by MedCalc Statistical Software version 16.4.3 (MedCalc Software, Ostend, Belgium). Considering the preliminary mean value of the primary end point (mu 9–29; sigma 6), to have a power of 90% with alpha of 0.5, a sample size of 4 patients (2 patients per group) could be sufficient. Plus, considering the preliminary mean value of the secondary end point for the photobiomodulation group (mu 8–4; sigma 2) having a power of 90% with alpha of 0.5, a sample size of 10 patients (5 patients per group) could be sufficient.

3. Results

3.1. Participants and Randomization

Between 1 January 2020 and 1 March 2021, 126 patients were screened for the study; 66 patients were deemed ineligible (Figure 2). Sixty patients were randomly assigned and consisted of the intention-to-treat population. Thirty of them were allocated to photobiomodulation and 30 were allocated to the placebo (laser kept switched off). All 60 patients received the randomly assigned treatment: photobiomodulation or placebo.

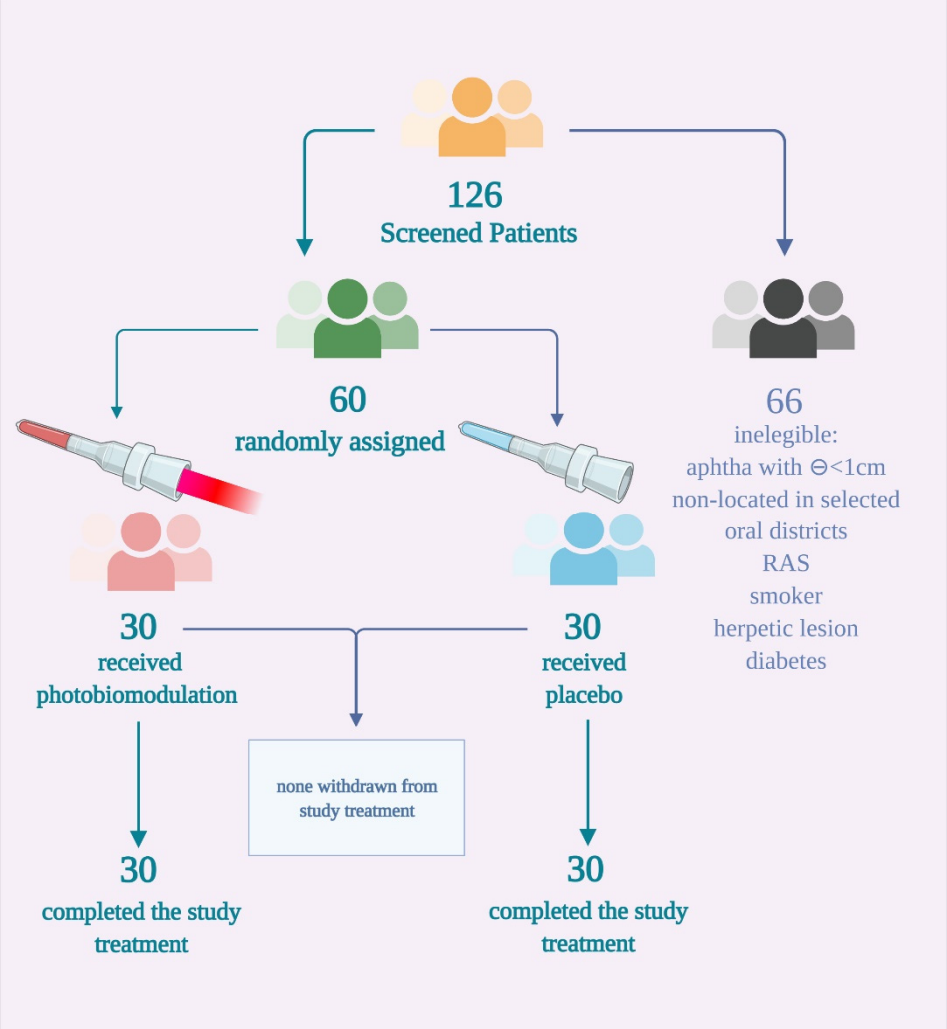

Figure 2. Design of the participants' randomization. Image created with BioRender.com. (accessed on 1 April 2021).

The photobiomodulation group was made up of Caucasians (61% females and 59% males) with a mean age ± standard deviation = 56.09 ± 10.7 (Figure 3). The placebo group was composed of Caucasians (55% females and 45% males) with a mean age ± standard deviation = 55.18 ± 12.5 (Figure 3). No statistical difference was observed concerning the age of the two groups ($p > 0.05$). The average diameter of the aphthae was 1.52 ± 0.27 cm and 1.48 ± 0.37 cm for the photobiomodulation and placebo groups, respectively. Plus, the average area of the aphthae was 1.38 ± 0.26 cm^2 and 1.35 ± 0.25 cm^2 for the photobiomodulation and placebo groups, respectively. No statistical difference was observed in the two groups concerning the aphthae diameter and area ($p > 0.05$). Lastly, according to the 4-point scale (range 0–3) based on the modified methods of Greer et al. [40] the aphthae of photobiomodulation and placebo group obtained an average score of 2.54 ± 0.52 and 2.63 ± 0.50, respectively; no statistical difference was observed between the two groups ($p > 0.05$).

3.2. Primary Variable

The analysis of the primary variable showed a highly significant difference between the two groups ($p < 0.05$) (Figures 4 and 5). Patients of the photobiomodulation group experienced complete healing in an average time of 8.13 days ± 1.69 (min 5–max 10 days), while for the placebo group, the average time extended to 30.76 ± 4.63 days (min 25–max 42 days).

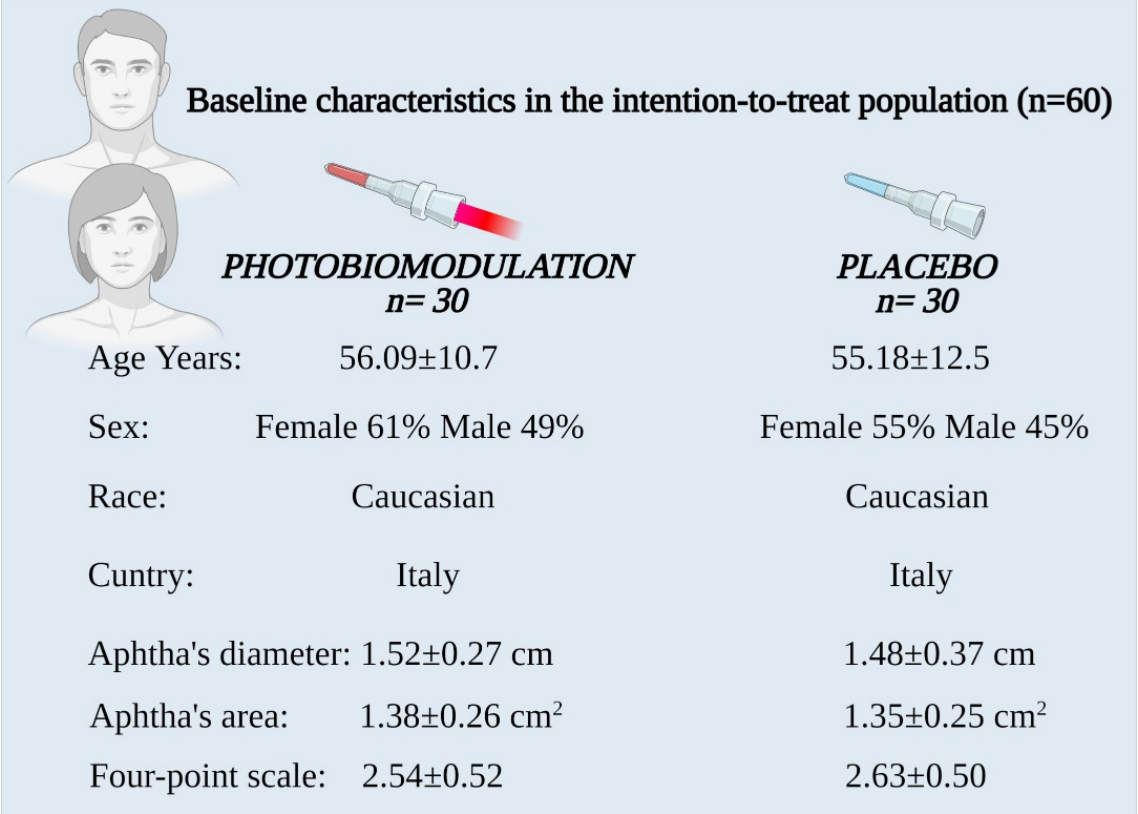

Figure 3. Characteristics of the randomized patients. Image created with BioRender.com. (accessed on 1 April 2021).

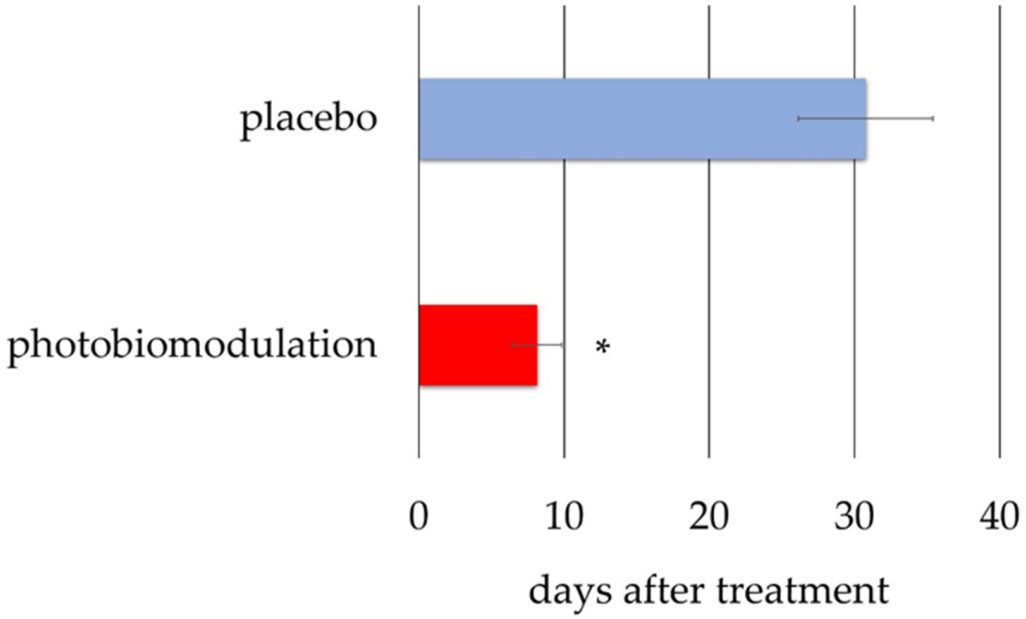

Figure 4. Effect of photobiomodulation and placebo treatment on aphtha's healing. Photobiomodulation therapy induced a statistical significative reduction of the healing time (* $p < 0.05$).

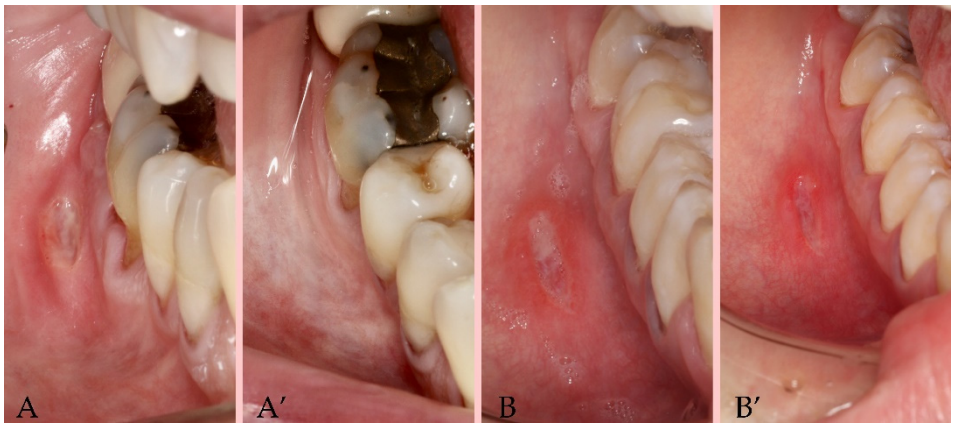

Figure 5. Effect of photobiomodulation (**A**,**A′**) and placebo (**B**,**B′**) treatment on aphtha's healing. (**A**–**B**), the aphtha before treatments. (**A′**–**B′**), recovery at 8 days after treatments. Photobiomodulation therapy induced complete healing at 8 days from the photobiomodulation treatment (**A′**), while, in the meantime, the patient treated with the placebo kept an evident and inflamed aphtha (**B′**).

3.3. Secondary Variable

The analysis of the second variable (Figure 6) showed no statistical difference between the photobiomodulation and placebo therapy groups before treatment ($p > 0.05$). Patients of the photobiomodulation therapy group experienced a statistically significant reduction in pain and discomfort 24 and 48 h after treatment ($p < 0.05$); the reduction was statistically higher ($p < 0.05$) 48 h after treatment compared to at 24 h.

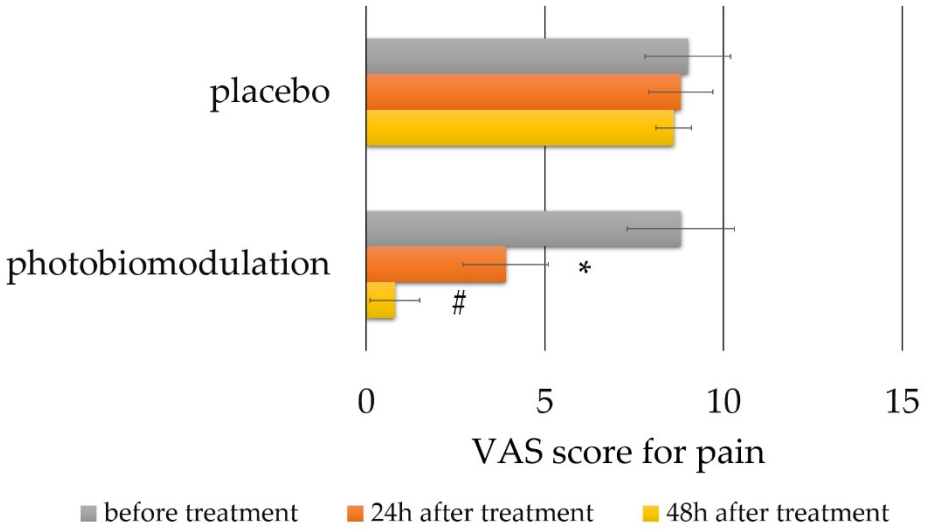

Figure 6. Effect of photobiomodulation and placebo treatment on pain. Photobiomodulation therapy induced a statistically significant reduction of the pain 24 and 48 h after photobiomodulation treatment. * $p < 0.05$ = before treatment vs. 24 h after treatment; # $p < 0.05$ = 24 h after treatment vs. 48 h after treatment. VAS = Visual Analogue Scale.

4. Discussion

Photobiomodulation therapy was significantly more effective than the placebo for the major aphtha treatment. Statistically, significant efficacy was shown in the primary end point; the aphtha healed faster, about 8 days, compared to the placebo group, which needed about 31 days. Moreover, the secondary end point, such as pain, was also significantly reached in the photobiomodulation group.

These clinical results support and are supported by our previous in vitro and preclinical evidence [32–38]. The photobiomodulation therapy through flat-top irradiation of 808-nm, and 1 W, 1 W/cm^2, 60 J/cm^2, CW induced an increment of oxygen consumption and ATP production in extracted mitochondria [32], in eukaryotic unicellular organisms [33,34], and in human endothelial cells [36]. Additionally, the effect on the homeostasis of calcium and the production of nitric oxide from a calcium–calmodulin dependent nitric oxide synthase enzyme was evidenced [42]. Studies on stem cells show that the therapy was significantly able to induce cell differentiation through a reduction in inflammation [38] and stimulation of biochemical/differentiating pathways involving key activators that regulate *de novo* actin polymerization and cytoskeletal rearrangement [37]. Preclinical evidence suggested that the therapy affected muscular and blood vessel contraction, decremented bacteria load, and tissue degeneration [35,43]. Reduction of inflammation markers and stimulated telomerase activity allowed us to speculate that a cell energized by photobiomodulation improves its proliferative potential thanks to the equilibrated recovery of homeostasis through the mitigating effect on both inflammation and tissue degeneration and a supportive effect on the energetic cellular metabolism.

This evidence can therefore explain the effect of photobiomodulation on our patients compared to the placebo.

Concerning the literature, our screening of papers did not show works focused on photobiomodulation and major aphtha management. However, a comparison with the effect of photobiomodulation on common aphtha healing ranks our clinical results in the supportive evidence.

Indeed, in the selected papers [20–31], the pain had mainly disappeared in an average time of 4.9 days. However, Jijin et al. [25] found a 71% pain reduction at 7 days, and Rocca et al. [27] showed a pain decrement from 81% to 16% in the same given period and in accordance with the photobiomodulation therapy employed. Additionally, the same papers showed that the healing process was completed in an average time of 6.3 days after photobiomodulation [20–31]. In the research by Soliman et al. [28], the healing was 97% by 7 days, and Jijin et al. [25] showed in the meantime an aphtha reduction of only 60%.

Additionally, photobiomodulation therapy seems to be helpful compared to drugs. Authors [23] completely recovered the pain over two days through 1064 nm irradiation or three days with 830 nm laser light, while the Solcoseryl adhesive paste (polidocanol anesthetic and antipruritic component) showed the same effect in 6 days; no synergic effect was found. Solcoseryl in association with the anti-inflammatory Granofurin was also employed by Lalabonova and Daskalov [26], who pointed out the disappearance of pain in only 55% of patients in 5 days against the 100% reduction obtained with the photobiomodulation in three days. In addition, authors [25], through Amlexanox oral paste (anti-inflammatory, antiallergic, immunomodulator product) administration showed less pain reduction (58%) than photobiomodulation treatment in three days.

Therefore, our results concur with the literature on the management of aphthae pain and healing with photobiomodulation by complying with the time of recovery. It is important to stress that our result is, however, obtained in the treatment of major aphthae, which have a longer recovery time compared to minor aphthae.

Our study has some limitations. First of all, the inclusion of only Caucasian patients limits the application of our therapy to only a part of the population; skin pigmentation can drastically influence photon energy absorption at 808-nm, inducing a dangerous thermal effect. Thus, the use of only the VAS scale for pain evaluation could influence bias.

However, our work also shows some strengths: first of all, we have used randomization, a double-blinded setup, and a sample size allowing an adequate study power. Additionally, the photobiomodulation and the placebo groups had similarities in terms of number, age, and sex distribution, as well as aphthae diameter and area, degree of erythema and exudation; however, no statistically significant difference was observed. Lastly, it is important to stress that the therapy parameters used in this article were extensively characterized in the previous work of our team on extracted mitochondria and in vitro and

preclinical experiments [17,32–38,42–44]. Basically, we pointed out their mechanisms of action and avoided their possible dangerous effects in terms of excessive oxidative stress and cell damage, supporting our results despite a short follow-up to our study.

5. Conclusions

In conclusion, our randomized, double-blind trial supports the effectiveness of photobiomodulation in the treatment of major aphthae. Irradiation of 808-nm and 1 W, 1 W/cm^2, 60 J, 60 J/cm^2 for 60 s on a spot-size area of 1 cm^2 accelerates the healing recovery and reduces pain compared to the patients treated with a placebo.

Author Contributions: Conceptualization, C.P. and A.A.; methodology, C.P., E.C., A.S. and A.A.; software, A.A.; validation, C.P., E.C., A.S. and A.A.; formal analysis, C.P., E.C., A.S. and A.A.; investigation, C.P., E.C., A.S. and A.A.; resources, S.B.; data curation, C.P., E.C., A.S. and A.A.; writing—original draft preparation, C.P., E.C., A.S. and A.A.; supervision, S.B.; funding acquisition, S.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study followed the Declaration of Helsinki with regards to medical protocol and ethics, and the Regional Ethical Review Board of DISC, University of Genoa approved the study (Unige-DISC-protocol number 0015098).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available upon request from the authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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