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ORIGINAL ARTICLE

Effects and parameters of the photobiomodulation in experimental models of third-degree burn: systematic review

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Abstract
This systematic review was performed to identify the role of photobiomodulation therapy in experimental models of third-degree burns used to induce oxidative stress. EMBASE, PubMed, and CINAHL databases were searched for studies published between January 2003 and January 2018 on the topics of photobiomodulation therapy and third-degree burns. Any study that assessed the effects of photobiomodulation therapy in animal models of third-degree burns was included in the analysis. A total of 17 studies were selected from 1182 original articles targeted on photobiomodulation therapy and third-degree burns. Two independent raters with a structured tool for rating the research quality critically assessed the articles. Although the small number of studies limits the conclusions, the current literature research indicates that photobiomodulation therapy can be an effective short-term approach to accelerate the healing process of third-degree burns, to increase and modulate the inflammatory process, to accelerate the proliferation of fibroblasts, and to enhance the quality of the collagen network. However, differences still exist in the terminology used to describe the parameters and the dose of photobiomodulation therapy.

Keywords Photobiomodulation therapy · Wound healing · Burns

Introduction

Severe burn injuries are the most traumatic and physically debilitating injuries affecting nearly every organ system and leading to significant morbidity and mortality. Early burn wound excision and skin grafting are common clinical practices that have significantly improved the outcomes of patients with severe burn injuries by reducing the mortality rate and the length of hospital stay. However, slow wound healing, infection, pain, and hypertrophic scarring continue to remain major challenges in burn research and management [1].

The first-degree burn is superficial and the lesion is located at the surface of the dermis. A second-degree also known as a partial superficial burn or a superficial dermis burn is when the lesion is located on the surface of the epidermis and the superficial papillary dermis and the upper area of the deep reticular dermis. In this type of burn, the pilosebaceous c complexes situated in the lower area of the deep reticular dermis remain intact. A deep burn or a third-degree burn affects the entire thickness comprising the epidermis, dermis, hypodermis and all cutaneous annexes, and, in some cases, even the adipose tissue [2].

Burn depth is related to the temperature and time exposed to the heat source. In animals and humans, a classical inverse relation exists between the temperature and time required to produce a specific degree of burn. It is worth noting that the critical temperature for thermal damage is about 43 °C—below which no damage occurs no matter how long the tissue is exposed to the source. This inverse relation indicates that one can create different degrees of thermal injury by either varying the time of exposure or varying the temperature of the heat source [3].

Burns are the most extensive forms of soft tissue injuries, occasionally resulting in extensive and deep wounds and death. Burns can lead to severe mental and emotional distress, because of excessive scarring and skin contractures [4].

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Healing is a complex process that involves a series of events, including clotting, inflammation, granulation tissue formation, epithelialization, collagen synthesis, and tissue remodeling. Thus, it has been extensively researched, particularly regarding the factors that could delay or hinder the healing process [5]. Third-degree burns have been a major focus of research and investigation, searching for new treatment methods in order to improve the care of burn patients and also provide greater speed for a satisfactory result without major functional and esthetic sequelae [4].

Low-level laser therapy (LLLT) has been recently used to stimulate the wound healing process [6]. Several effects of LLLT have been claimed to induce this phenomenon, including increased ATP production and increased mitochondrial membrane potential [7]. Several investigators reported that photobiostimulation of the wound healing process stimulates fibroblast proliferation; significantly increases re-epithelialization, collagen synthesis, and granulation tissue formation; accelerates wound closure; improves tensile strength of the scars; and determines faster healing of burns [8].

However, the cellular mechanisms of photobiomodulation therapy (PBMT) using LLLT are not well understood despite much discussion on these mechanisms in the literature. Controversial results are reflective of the complexity of the appropriate parameter selection before each treatment session. Given the lack of uniform parameters in the literature, the translation of clinical control studies is still incipient. In view of the above considerations, this systematic review aimed to assess and discuss the parameters and results obtained in experimental studies performed on third-degree burn models and to verify based on these results the uniformity of expected effects and ideas for a possible translation of preclinical studies to randomized clinical trials.

Materials and methods

Search strategy

A systematic search of studies published between 2003 and January 2018 was performed in three electronic databases: EMBASE (Excerpta Medica Database), PubMed (Public/Publisher MEDLINE), and CINAHL (Cumulative Index to Nursing and Allied Health Literature). First, keywords were selected from related articles. MeSH and Scopus international data lines were used to find more related keywords with close meanings: ("low-level light therapy" [MeSH Terms] OR ("low-level" [All Fields] AND "light" [All Fields] AND "therapy" [All Fields]) OR "low-level light therapy" [All Fields] OR "lllt" [All Fields]) AND ("wound healing" [MeSH Terms] OR ("wound" [All Fields] OR "repair" [All Fields]) AND ("burns" [MeSH Terms] OR "burns" [All Fields] OR "burn" [All Fields]) AND third [All Fields] AND degree [All Fields] AND ("rats" [MeSH Terms] OR "rats" [All Fields]). The search was repeated after the review of the eligible papers to specifically search for experimental methodologies and outcomes and parameters of photobiomodulation. In addition, we reviewed the retrieved articles to identify possible additional studies (Fig. 1).

Study selection

We examined the title list and abstracts identified by the literature searches for potentially relevant studies. Two independent reviewers (SAS and CO) applied predetermined inclusion criteria to all studies. Conflicts were resolved by a third independent researcher (PTC).

The following inclusion criteria were applied:

1. Live animal subjects
2. Experimental studies performed on third-degree burn models
3. Random allocation of treatment
4. Photobiomodulation provided as an intervention to at least one treatment group
5. A quantitative or semi-quantitative assessment
6. English language

Abstracts were reviewed by at least two raters to determine if they met the eligibility criteria.

The following exclusion criteria were applied:

1. In vitro studies
2. Clinical studies and systematic review articles with or without meta-analysis
3. Papers not published in English language
4. Microbiological studies

Assessment of study quality

Potentially eligible articles were printed, reviewed, and critically appraised for quality rating by two independent reviewers. Systematic reviews are commonly performed in human research, but rarely in animal research. To assess the quality of included studies, we used SYRCLE's RoB [9] tool that contains 10 items to investigate any important sources of bias such as allocation, adjust for confounder, assignment to the different groups adequately concealed during, animals randomized for housed, blinded investigators, animals random selection for outcome assessment, blinded outcome assessor, addressing incomplete outcome, and free of selective outcome reporting and other risk of bias (Fig. 2).

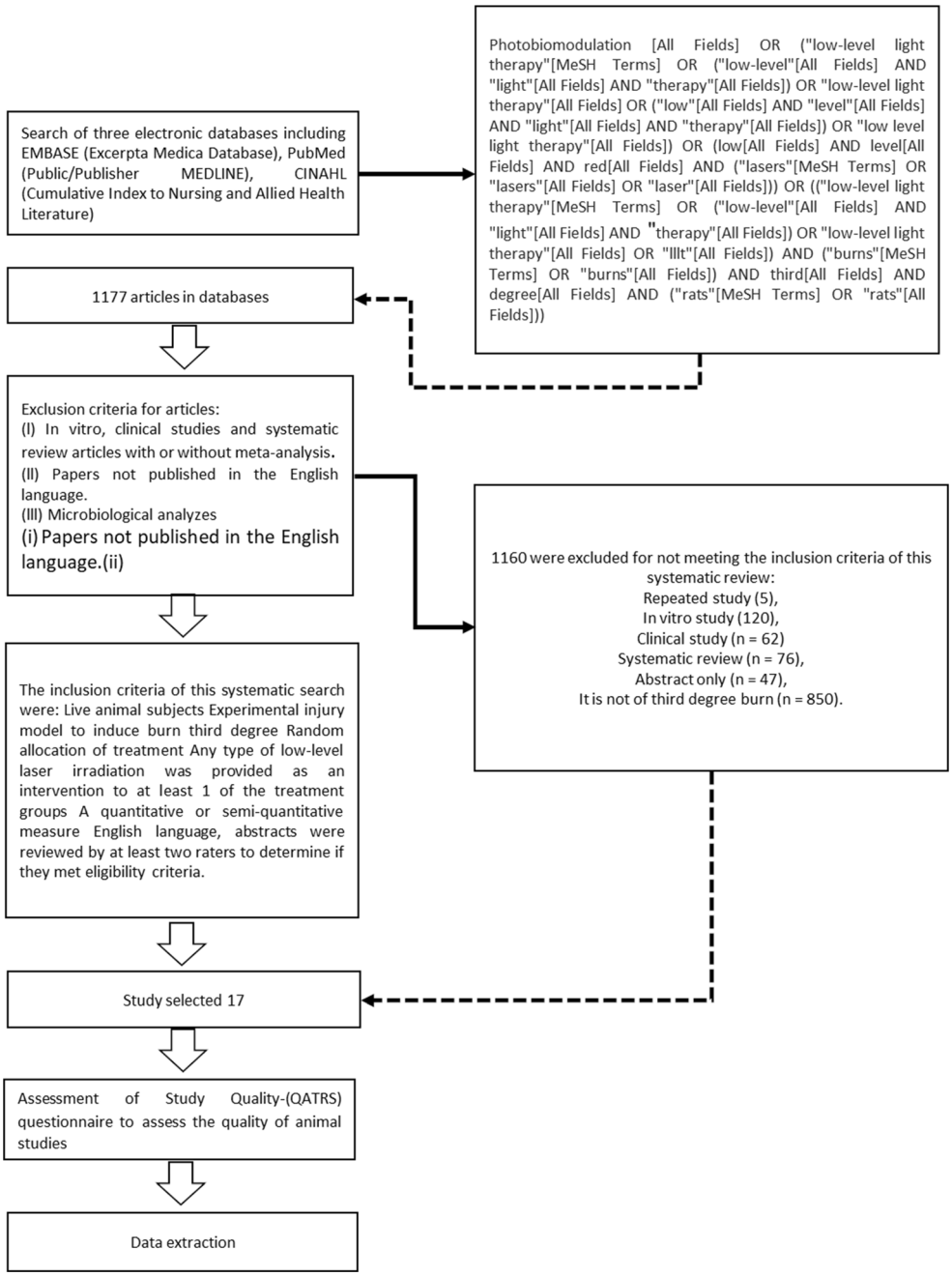

Fig. 1 Flow chart of experimental design

Results

We detected 1182 articles in the databases. Of these, 1164 were excluded for not meeting the inclusion criteria of this systematic review: repeated study ($n = 5$), in vitro study ($n = 120$), clinical study ($n = 62$), systematic review ($n = 76$), abstract only ($n = 47$), it is not of third-degree burn ($n = 854$). Finally, 17 studies [4, 10–25] in which diverse treatment parameters of injuries were assessed were included for critical evaluation of the effectiveness of PBMT in third-degree burn. The 17 selected studies totaled a sample of 845 animals and showed some common characteristics, namely the use of adult male Wistar rat with a mean weight of 278.5 g as an experimental model and the dorsal region of the animals as the region where burns were performed in all (100%) studies. The burn model was created using a cylinder connected to boiling water (27.7% of cases) [14–18, 20], a heated metal (22.2%) [4, 10–13, 16, 22]. Instrument heated until red and incandescent (11. 1%) [24, 25] immersion in water at 95 °C (5.5%) [15], immersion in water at 100 °C (5.5%) [23], or cylindrical brass rod cooled to 77 K (5.5%) [21]. These findings suggested a good standardization between the experimental models of third-degree burns, allowing the reproducibility of the experimental models (Table 1).

Type of bias	Domain	Description of domain	Review authors judgment
Selection bias	Sequence generation	Describe the methods used, if any, to generate the allocation sequence in sufficient detail to allow an assessment whether it should produce comparable groups.	Was the allocation sequence adequately generated and applied? (*)
Selection bias	Baseline characteristics	Describe all the possible prognostic factors or animal characteristics, if any, that are compared in order to judge whether or not intervention and control groups were similar at the start of the experiment.	Were the groups similar at baseline or were they adjusted for confounders in the analysis?
Selection bias	Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrolment.	Was the allocation adequately concealed? (*)
Performance bias	Random housing	Describe all measures used, if any, to house the animals randomly within the animal room.	Were the animals randomly housed during the experiment?
Performance bias	Blinding	Describe all measures used, if any, to blind trial caregivers and researchers from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.	Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?
Detection bias	Random outcome assessment	Describe whether or not animals were selected at random for outcome assessment, and which methods to select the animals, if any, were used.	Were animals selected at random for outcome assessment?
Detection bias	Blinding	Describe all measures used, if any, to blind outcome assessors from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.	Was the outcome assessor blinded?
Attrition bias	Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized animals), reasons for attrition or exclusions, and any re-inclusions in analyses for the review.	Were incomplete outcome data adequately addressed? (*)
Reporting bias	Selective outcome reporting	State how selective outcome reporting was examined and what was found.	Are reports of the study free of selective outcome reporting? (*)
Other	Other sources of bias	State any important concerns about bias not covered by other domains in the tool.	Was the study apparently free of other problems that could result in high risk of bias? (*)

Fig. 2 Representation of the SYRCLE's risk of bias tool for animal studies. Hooijmans et al. (2014) [9]

A great variation of performed analyses and dependent variables was found in the selected studies; although the majority (50.0%) of them used different methods to assess the effect of PBMT in several phases of the repair process [4, 10–14, 16, 20–25], some studies (5.5%) focused on bacterial infection (Table 2) [15].

Table 3 shows the parameters used in the selected studies. Whereas 72.2% of studies used a laser with a wavelength in the red spectrum, some authors chose a 632.8-nm laser [20, 21]; others used a laser with a wavelength in the range of 660 nm [4, 10–12, 14, 15, 18, 19, 22], 685 nm [17], and 640 nm [13, 23], and 5.5% used a laser with a wavelength of 890 nm [16], 11.1% used a laser with a wavelength of 780 nm [18, 19, 22], 11.1% used a laser with a wavelength of 400 nm [24, 25], 5.5% used a laser with a wavelength of 520 and 550 nm [22], and used a laser with a wavelength of

Table 1 Study the characteristics of selected controlled experimental studies of photobiomodulation therapy effects on third-degree burn

Authors	Animal type	Gender (M/F)	Animal race	Age (months)	Weight (g)	Induction model	temperature/ exposure time	Site Injury	Ethics committee
Gomes 2017 [4]	Rat	Female	Wistar	–	± 300 g	hot water at 60 °C	45 s	Dorsum	Not
Brassolati 2016 [10]	Rat	Male	Wistar	12 weeks	± 280 g	Aluminum plate 150 °C	10 s	Dorsum	Yes 022/2013
Trajano 2014 [11]	Rat	Male	Wistar	–	250–350 g	Metal rod. 80 °C	15 s	Dorsum	Yes
Fiorio 2014 [12]	Rat	Male	Wistar	–	260 ± 20 g	Aluminum plate 120 °C	5 s	Dorsum	Yes A107 /CEP/2007
Fiorio 2011 [13]	Rat	Male	Wistar	–	260 ± 20 g	Aluminum plate 120 °C	5 s	Dorsum	Yes A107/ CEP/2007
Nuñez 2013 [14]	Rat	Male	Wistar	Adult	≅ 300 g	Rubber tube connected boiling water	5 s	Dorsum	Yes
Moraes 2013 [15]	Rat	Male	Wistar	–	300–350 g	Immersion in water at 95 °C	14 s	Dorsum	Yes 098/2009
Khoshavaghit 2011 [16]	Rat	Male	Wistar	4 months	300 g	Cylinder connected boiling water	2 s	Dorsum	Yes
Garcia 2010 [17]	Rat	Male	Wistar	Adult	180–220 g	Heated punch 80 °C	30 s	Dorsum	Yes
Meireles 2008 [18]	Rat	Male	Wistar	Young adult	200–230 g	Instrument heated	20 s	Dorsum	Yes
Meireles 2008 [19]	Rat	Male	Wistar	Young adult	200–230 g	Instrument heated	20 s	Dorsum	Yes
Bayat 2008 [20]	Rat	Male	Wistar	Adult	250 ± 30 g	Cylinder connected boiling Water	7 s	Dorsum	Yes
Da Silva D de F 2006 [21]	Rat	Male	Wistar	Adult	300 g	cylindrical brass rod cooled to 77 K	2 sequences of 5 s Repeated 3 days	Dorsum	Not
Catão 2015 [22]	Rat	Male	Wistar	–	200–250 g	Iron heated	20 s	Dorsum	Yes 0019/240712
Neves 2014 [23]	Rat	Male	Wistar	–	250 ± 25	tube with water heated to 100 °	20 s	Dorsum	Yes 496/2008
Oliveira 2010 [24]	Rat	Male	Wistar	Adult	200–230 g	instrument heated until red and incandescent	20 s	Dorsum	Yes
Oliveira 2011 [25]	Rat	Male	Wistar	Adult	200–230 g	instrument heated until red and incandescent	20 s	Dorsum	Yes 013/06

Table 2 Study the characteristics (sample sizes, number of groups, number of animals/group, dependent variables) of selected experimental studies of controlled animals on effects of photobiomodulation therapy on third-degree burn

Authors	Sample sizes	Number of groups	Number of animals/group	Dependent variables
Gomes 2017 [4]	12	3	4	Morphological analysis (inflammatory response; granulation tissue; presence or absence of hair follicles; presence or absence of ulcers; analysis of the collagen organization)
Brassolati 2016 [10]	30	3	10	Histopathological Analysis; Blood Vessel Morphometry; Morphometry of Collagen Fibers; Immunohistochemistry.
Trajano 2014 [11]	18	3	6	Total RNA extraction; complementary DNA synthesis.
Fiorio 2014 [12]	48	4	12	Morphological analysis (histologic analysis, morphometric analysis) count the inflammatory cells, type of collagen fibers.
Fiorio 2011 [13]	24	4	6	Histological analysis (measured at the skin surface); count the inflammatory cells.
Nuñez 2013 [14]	36	2	18	Histomorphometrical analysis; quantitative assessment of new vessels; leukocyte differential counting; Laser doppler flowmetry.
Moraes 2013 [15]	36	3	12	Macroscopic evaluation; morphometric evaluation; microscopic evaluation; mesoscopic analysis.
Khoshavaghit 2011 [16]	48	2	24	Morphometric examination (histologic examination) numbers of types 1, 2, and 3 mast cells, and the total number of mast cells in 100 zones of burned skin.
Garcia 2010 [17]	96	4	24	Histological analysis by light microscopy
Meireles 2008 [18]	55	3	15/20	Microscopy analysis
Meireles 2008 [19]	55	3	15/20	Microscopy analysis
Bayat 2008 [20]	60	4	15	Morphometrical examination (histologic examination numbers of types 1, 2, and 3 mast cells, and the total number of mast cells in 100 zones of burned skin)
Da Silva D de F 2006 [21]	20	5	4	Collagen birefringence
Catão 2015 [22]	100	5	20	Histological processing; morphological aspects of inflammatory cells and collagen fibers; Quantitative analysis of the collagenization area
Neves 2014 [23]	72	6	12/6	Digital photogrammetry—macroscopic analysis; Histomorphometric analysis.
Oliveira 2010 [24]	45	3	15	Histological analyses
Oliveira 2011 [25]	90	18	5	Histological analysis

2000 nm [24, 25]. In relation to the power of the lasers, a very large variation was detected, ranging from 10 mW [20, 21] to 100 mW [10, 11]; the intermediate powers were as follows: 75 mW [16], 40 mW [22], 50 mW [10], 35 mW [12, 18, 19], 30 mW [13, 14], 20 mW [15], 5 mW [4], 0.05 mW [17], 0.11 mW [23], 3.7 mW [24], and 0.95 W [25]. Other important parameters of PBMT were also obtained in this analysis: energy (only 27.4% of studies reported this parameter [10, 12, 13, 22, 23]), beam area (66.6% of reviewed studies assessed this parameter [4, 10, 12–17, 20, 23–25]), exposure time to PBMT (72.2% of studies reported it [4, 10, 12–14, 16, 17, 20–25]), and the density of power (55.5% of papers described it [4, 10, 12, 14–17, 21, 24, 25] (Table 3)). Regarding the effect of PBMT, 22.2% of studies reported statistically significant positive effects, 50.0% of studies reported positive but not statistically significant effects, and 22.2% % of studies reported partially positive effects (Table 4).

Risk of bias and quality of included studies

Risk of bias was assessed using the SYRCLE Risk of Bias Tool for all 17 studies that met inclusion criteria for our review. None of the experiments were judged to be low risk of bias across all domains. All studies reported similar experimental and control groups at baseline, which reduces the risk of selection bias based on animal characteristics. Despite stating that allocation of subjects to experimental and control groups was random, only one [17] of the studies explicitly described the method of random

Table 3 Study the parameters of photobiomodulation therapy in third-degree burn

Authors	Wave length (nm)	Energy density (J/cm^2)	Energy (J)	Power density (W or mW/cm2)	Spot size (cm^2)
Gomes 2017 [4]	660 ± 2 nm	1 J/cm^2		6.25 mW/cm^2	0.04 cm^2/0.8 cm^2
Brassolati 2016 [10]	660 nm	12.5/25 j/cm^2	0.5/1 j	1.25/2.5 mW/cm^2	0.04 cm^2
Trajano 2014 [11]	660 nm	20 j/cm^2	–	–	–
Fiorio 2014 [12]	660 nm	3/4 j/cm^2	2.1/2.8 j	0.05 W/cm^2	0.63 cm^2
Fiorio 2011 [13]	640/23 nm	4 j/cm^2	–	–	0.05 cm^2
Nuñez 2013 [14]	660 nm	1/4 j/cm^2	–	30 mW/cm^2	1 cm^2
Moraes 2013 [15]	660 nm	3/6 j/cm^2	–	35 mW	0.035 cm^2
Khoshavaghit 2011 [16]	890 nm	0.924 j/cm^2	–	1.08 mW/cm^2	1 cm^2
Garcia 2010 [17]	685 nm	4.5 j/cm^2	–	0.5 W/cm^2	0.01 cm^2
Meireles 2008 [18]	660/780 nm	20 J/cm^2	–	–	–
Meireles 2008 [19]	660/780 nm	20 J/cm^2	–	–	
Bayat 2008 [20]	632.8 nm	38.2/76.4 j/cm^2	–	–	3.14 mm^2
Da Silva D de F 2006 [21]	632.8 nm	1 J/cm^2		6 mW/cm^2	
Catão 2015 [22]	660/780/660/520/550 nm	10/60 J/cm^2	0.4/0.6 J		
Neves 2014 [23]	640 ± 20 nm	4/16 J/cm^2	4.51/74.8 J		1.13/4.67 cm^2
Oliveira 2010 [24]	400/2000 nm			40 mW/cm^2	23.7 cm^2
Oliveira 2011 [25]	400/2000 nm	20/40 J/cm^2–10.2/20.4 J/cm^2		0.04 W/cm^2	23.7cm^2

Authors	Irradiation time per point (sec)	Duration of treatment (days)	Frequency of treatments (days)	Laser frequency (Hz)	Distance irradiance	Power (mW or W)
Gomes 2017 [4]	160 s	12 days	0, 5, 12 days	–	–	5 mW
Brassolati 2016 [10]	10 s	8 days	2, 4, 6, 8 days	–	Contact	50/100 mW
Trajano 2014 [11]	–	5 days	Daily	–	–	100 mW
Fiorio 2014 [12]	60/80 s	4/8 days	48 h intervals	–		35 mW
Fiorio 2011 [13]	66 s	7, 15 days	48-h intervals		–	30 mW
Nuñez 2013 [14]	33/133 s	10 days	1, 3, 8, 10 days	–	Contact	30 mW
Moraes 2013 [15]	–	–	Three times per week	–	–	20 mW
Khoshavaghit 2011 [16]	856 s	20 days	Three times per week at 56-h intervals	80 Hz	–	75 W
Garcia 2010 [17]	81 s	3, 7, 14 days	–	–	Contact	0,05 W
Meireles 2008 [18]	–	21 days	3, 5, 7, 14, and 21 day	–	–	35 mW
Meireles 2008 [19]	–	21 days	3, 5, 7, 14, and 21 days		–	35 mW
Bayat 2008 [20]	120/240 s	7, 16, 30 days	Daily	–	Contact	10 mW
Da Silva D de F 2006 [21]	3 min	3, 7, 10, 14 and 17 days				10 mW
Catão 2015 [22]	5 min.	3, 7, 14, 21 days	Daily			40/60 mW
Neves 2014 [23]	41/680 s.	7, 14 days	48-h intervals		2.44 cm	0.11 mW
Oliveira 2010 [24]	255/510 s.	7, 14, 21 days	Daily		10 cm	3.7 mW
Oliveira 2011 [25]	255/510 s	7, 14, 21 days	Daily		10 cm	0.95 W

sequence generation. For this reason, risk of bias in the sequence generation domain was judged as "High risk of bias" in 94.1% of studies. One result that should be emphasized is that 100% of studies do not adequately described the method used to conceal allocation. Only two [10, 17] of the studies stated that animals were randomly housed. One hundred percent of studies do not reliably report blinding of caregivers and investigators from knowing which intervention each animal received. Only 5.8% [4] of studies reported random outcome assessment, but 100% of studies were not documented with the blinding of the outcome assessor. Also it was not clear in 100% of the studies if the incomplete outcome data adequately addressed, i.e., if they were reported that all animals were included in the analysis or even what were the reasons for missing outcome data unlikely to be related to true outcome? (e.g., technical failure). Using the signaling questions provided, all studies were rated as low risk of attrition and reporting bias. Furthermore, we did not identify any additional sources of bias not already

Table 4 Classification according to the type of results found in the studies of selected experimental on effects of photobiomodulation therapy on third-degree burn

Authors	Positive effects: statistically significant	Positive effects: not significant	Partial effect statistically significant	No effect
Gomes 2017 [4]			X	
Brassolati 2016 [10]	X			
Trajano 2014 [11]	X			
Fiorio 2014 [12]	X			
Fiorio 2011 [13]			X	
Nuñez 2013 [14]			X	
Moraes 2013 [15]	X			
Khoshavaghit 2011 [16]			X	
Garcia 2010 [17]			X	
Meireles 2008 [18]		X		
Meireles 2008 [19]		X		
Bayat 2008 [20]			X	
Da Silva 2006 [21]			X	
Catão 2015 [22]			X	
Neves 2014 [23]			X	
Oliveira 2010 [24]		X		
Oliveira 2011 [25]		X		

covered by the SYRCLE Risk of Bias Tool, such as industry funding, conflict of interest, or failure to publish in a peer-reviewed journal. With regard to journals where articles were published, 100% are indexed and found in journals with impact factor ranging from 0.931 to 2.7. Of note, none of these studies documented a calculation for sample size (Table 5 Figs. 3 and 4).

Discussion

In this review, studies that mainly focused on the effects of PBMT on experimental lesions caused by third-degree burns were analyzed. No unanimity was detected regarding the used experimental technique or regarding the methods used to measure obtained results. Frequently, different classifications and

Table 5 Assessment of study quality (Quatrs) and Journal and impact factor where selected experimental studies of photobiomodulation therapy on third-degree burn were published

Authors	Journal	Impact factor
Gomes 2017 [4]	Revista da Associação Médica Brasileira	0.931
Brassolati 2016 [10]	Microscopy Research and Technique	1.147
Trajano 2014 [11]	Lasers in Medical Science	2.229
Fiorio 2013 [12]	Lasers in Medical Science	2.229
Fiorio 2011 [13]	Journal of Cosmetic and Laser Therapy	1.113
Nuñez 2012 [14]	Lasers in Medical Science	2.229
Moraes 2012 [15]	Lasers in Medical Science	2.229
Khoshavaghit 2011 [16]	Photomedicine and Laser Surgery	1.680
Garcia 2009 [17]	Lasers in Medical Science	2.229
Meireles 2008 [18]	Photomedicine and Laser Surgery	1.680
Meireles 2008 [19]	Photomedicine and Laser Surgery	1.680
Bayat 2008 [20]	Journal Rehabilitation Research & Development	1.277
Da Silva 2006 [21]	Journal of Biomedical Optics	2.700
Catão 2015 [22]	Lasers in Medical Science	2.229
Neves 2014 [23]	Lasers in Medical Science	2.229
Oliveira 2010 [24]	Photomedicine and Laser Surgery	1.680
Oliveira 2011 [25]	Photomedicine and Laser Surgery	1.680

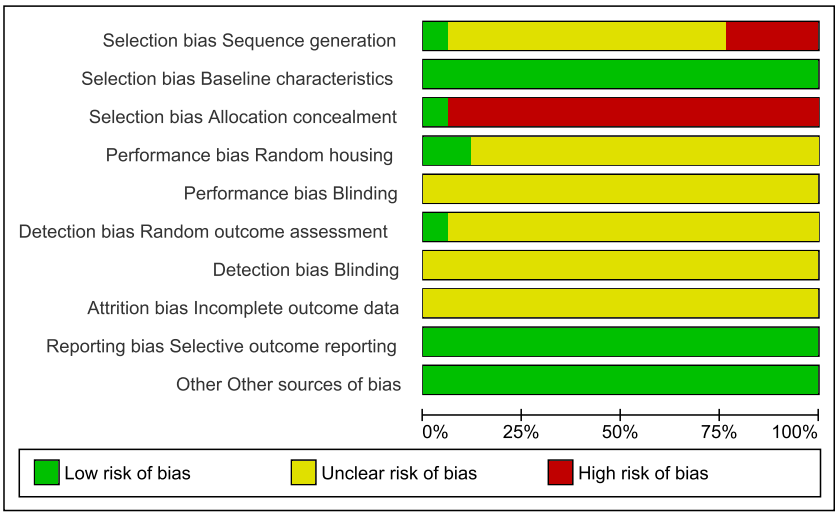

Fig. 3 Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

evaluations were used to designate similar variables. This fact may be related to different analyzed processes, such as healing, inflammation, or even isolated stages of the healing process. Enwemeka et al. [26] stated that such failures are the cause of inconsistencies in the literature, especially regarding the application of PBMT. Regarding the used models, it should be noted that the burn itself can vary according to used animal for experimentation, as well as according to the time and the method the animal is exposed to heat or even chemical. The most common etiologies requiring care in a burn center are fire/flame (43%), followed by scalds (34%), contact with hot objects (9%), electricity (4%), and chemical agents [27].

A search for an ideal experimental animal model is important for burn research. In our review, we observed that 100% of the evaluated studies used the Wistar rats as the animal model, which is in agreement with what seems to be the most practical and widely used model in other studies. Animal models can replace direct testing in human beings, especially when the toxicity of the test material is unknown. New Zealand rabbits, Sprague-Dawley rats, Wistar albino rats, BALB/c mice, and pigs have been used as animal models in burn research; rats are the most commonly used of all these animals [28].

The form that was used to trigger the burn has an important role in the type of injury and complications triggered after the burn. Of 17 analyzed articles, 23.5% [14, 15, 17, 18] used cylindrical metal systems connected to hot water and 41.1% [4, 10–13, 16, 20] used a heated metal to produce burns; only a small percentage used immersion in hot water [15]. According to Guo et al. [28] and Venter et al. [29], temperature, duration, and contact pressure are the three primary variables needed to achieve a uniform burn. However, these three factors varied greatly or even have not been described in some evaluated studies, indicating a lack of standardization and uniformity in burns induction.

In evaluated studies, a series of analyzed variables were focused on tissue repair (100%) [4, 10–25] and different methodologies were used to evaluate the action of PBMT in various phases of tissue repair. These results demonstrate that most studies still consider that PBMT affect tissue repair (collagen deposition and proliferation of fibroblasts and neoformation of blood vessels) and modulate the inflammatory process. Moreover, some of these studies were focused on the interference of the infection process in the healing of burns. However, we believe that there is a lack of investigations regarding the role of PBMT on inflammatory mediators and oxidative stress following this type of burn, given the evidence in the literature on the action of PBMT and modulation of inflammatory biomarkers [30] and the increase of antioxidant substances [31, 32] during the use of PBMT.

Burn injuries result in various local and systemic responses. In response to injury, a local and systemic release of inflammatory mediators, reactive oxygen species, and reactive nitrogen species occurs, which is often confounded with local infection. Among the circulating vasoactive and inflammatory mediators are the histamines, prostaglandins, kinins, platelet aggregation factors, angiotensin II, vasopressin, and corticotropin-releasing factors, and cell signaling proteins, such as cytokines and chemokines [27].

The focus of the current review was to assess the parameters of LLLT used during PBMT to improve the healing and inflammatory process in third-degree burns. In this respect, we realized that there is agreement regarding the type of wavelength used in studies, with wavelength ranging from red (632.8 nm;[20, 21] 640 nm; [13, 23] 660 nm; [4, 10–12, 14, 15, 18, 19, 22] and 685 nm [17] nm) to the infrared (780 nm; [18, 19, 22], 890 nm; [16]) and 2000 nm [24, 25]spectrum (82.3% of the studies opted for PBMT operating in the red band). Such an option is because of the depth of the structures to be stimulated, as, according to Oliveira Silva et al. [32], the effective penetration of the tissue by the light and the specific

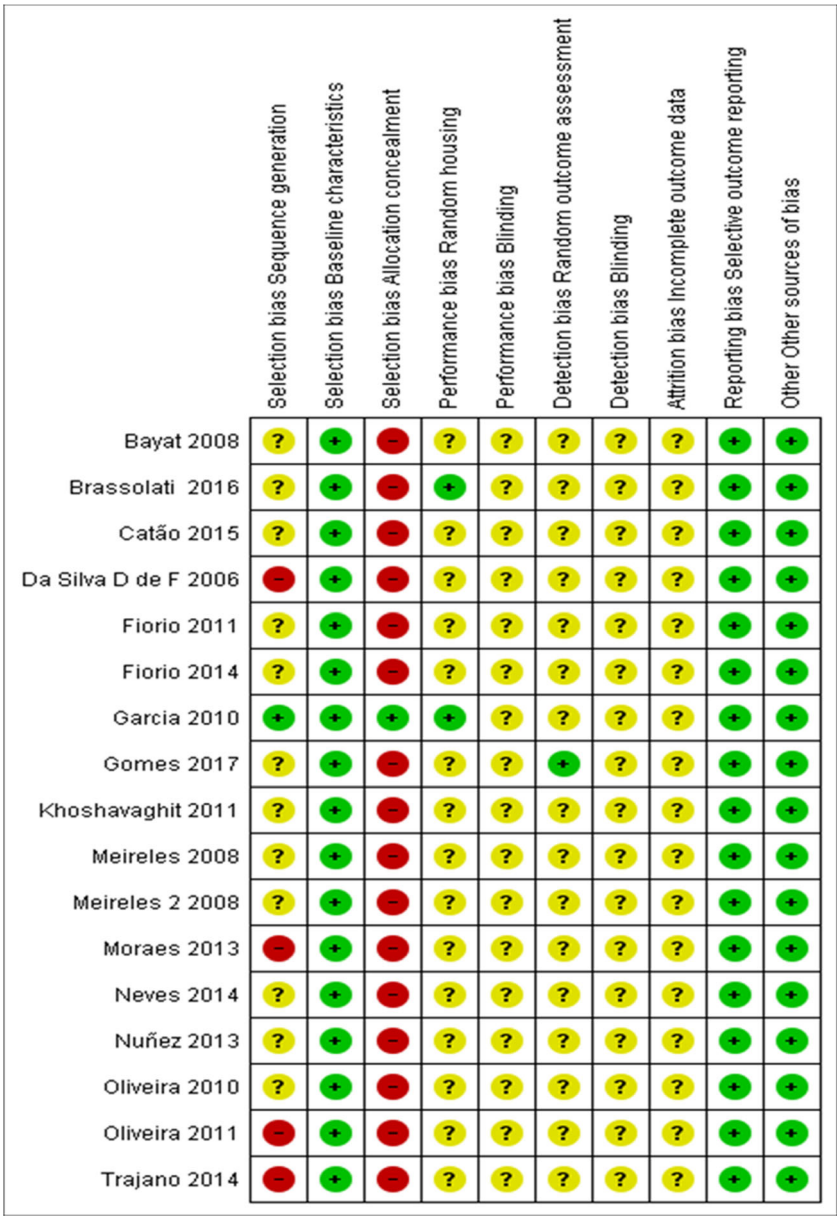

Fig. 4 Risk of bias summary: review authors' judgements about each risk of bias item for each included study

light wavelength absorbed by the photoacceptors are two of the main parameters to be considered in light therapy. In the fabric, there is an *optical window* running from approximately 650 to 1200 nm, where effective tissue penetration into light is maximized.

In relation to the power of the lasers, a very large variation was detected, ranging from 10 to 100 mW. Few studies reported the energy, beam area, exposure time, and power density of used lasers. We consider the lack of these parameters a great failure, hindering or preventing the reproducibility of the studies. The absence of these parameters weakens the studies once the literature has shown that the results of PBMT depend on the irradiation time and used dose. If we take into account the fact that different areas of beam and powers need different irradiation times and energy densities, the reproducibility of these studies is threatened [33].

PBMT has demonstrated positive effects in stimulating cellular activities involved in the wound healing process. The action of PBMT is based on the absorption of light by the tissues, which generates modifications in cellular metabolism and alter the exchange of calcium through the cell membrane. These PBMT-promoted alterations may enhance the synthesis of DNA, RNA, and cell cycle regulatory proteins, stimulating cell proliferation and connective tissue reestablishment during tissue repair and wound healing [34].

The small number of studies for third-degree burns does not allow us to affirm that there is clear scientific evidence of the

benefits of PBMT in this type of burn. Bjordal et al. [35] reports that evidence for most interventions lack sufficient statistical power to make valid conclusions. However, on the other hand, if we follow the results presented by the studies included in this review, we may suggest that photobiomodulation is an effective short-term approach to accelerate the healing of wounds caused by third-degree burns, modulate the inflammatory process, increase deposition of type I and III collagen, and downregulate matrix metalloproteinases.

Moreover, the parameters used for PBMT in the examined studies, such as laser output power, irradiation distance, irradiation frequency per day, number of treatment sessions, irradiated energy per day, and total irradiated energy, did not meet the current recommendations for reproducible studies. (Inflammatory phase, proliferation and remodeling) and modulation of the inflammatory process (chemokines, growth factor cytokines), in third-degree burns. In conclusion, it is important to note that PBMT is an effective short-term approach for third-degree burns. However, the lack of uniformity in the terminology used to describe parameters and the dose used for PBMT limits the ability to reach firm conclusions.

According to Khatib et al. (2015) [36], in general, animal studies in comparison to human RCTs have low internal validity as well as standard practice to randomize allocation of the animal to the intervention and control arms and to blind the investigators and outcome assessors. However, some systematic reviews of animal studies show that a similar effect of an intervention can be found over a number of species/strains which suggests that there is a high probability that it can be extrapolated to humans. The quality of the included studies varied widely; none of the included studies exhibited an overall low risk of bias in all criteria analyzed by the SYRCLE Tool, while the bulk of the remaining experiments ranged in the unclear risk of bias. This fact is rather sobering, when placed in the context of potential translational applications of the evaluated interventions. De Vries et al. [37], describes the importance of systematic reviews of animal experimental studies as a precursor for the implementation of subsequent preclinical and clinical studies. However, there is a problem in systematic review of animal studies. Negative results are often not published, leading to publication bias.

In conclusion, it is important to note that PBMT is an effective short-term approach for third-degree burns. However, the lack of uniformity in the terminology used to describe parameters and the dose used for PBMT and the risk of vies in the methodological conduction of some studies limits the ability to reach firm conclusions.

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Compliance with ethical standards

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

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