Lasers in Medical Science (2019) 34:1725–1734
https://doi.org/10.1007/s10103-019-02813-5

REVIEW ARTICLE

Photobiomodulation effects on keratinocytes cultured in vitro: a critical review

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Received: 27 February 2019 / Accepted: 21 May 2019 / Published online: 1 June 2019

Abstract

Photobiomodulation therapy (PBMT) has been widely used for the promotion of tissue repair. Despite these therapeutic benefits, in some cases, PBMT appears to be unsuccessful, and the strongest hypothesis is that this failure is due to inadequate light dosimetry and wavelengths. The objective of the present critical review was to evaluate the effects of PBMT on cultured keratinocytes using blue, red, or near-infrared light categorized into arbitrary ranges of energy density (0.1–5.0, 5.1–10.0, 10.1–15.0, and over 15.0 J/cm^2). The electronic searches were conducted in PubMed, Web of Science, Scopus and LILACS databases, and included LASER or LED devices. A total of 55 articles evaluating the effects of PBMT on cell viability, proliferation, migration, and cytokine and growth factor production were included. Overall, the studies failed to provide detailed information about light dosimetry or detailed experimental conditions. The vast majority of the energy densities tested produced unmodified results regardless of the wavelength applied. However, it was possible to observe that red and near-infrared light had more stimulatory effects than blue light. In addition, for all parameters analyzed, favorable outcomes were mostly obtained in the range of 0.1–5.0 J/cm^2. The less explored energy densities were within the 10.1–15.0 J/cm^2 range. Energy densities above 15.0 J/cm^2 were ineffective or tended to cause cell death. The heterogeneity of the data does not allow us to define a PBMT range setting protocol that would have beneficial effects on keratinocytes.

Keywords Photobiomodulation therapy · Keratinocytes · Wound healing · Dosimetry

Introduction

Photobiomodulation therapy (PBMT), characterized by the use of light at low power, has been widely used for therapeutic purposes in order to produce analgesia, immunomodulation, wound healing, and tissue regeneration [1–4]. In fact, photon absorption modifies the molecular configuration of one or more photoacceptors, leading to an alteration of their own properties with possible consequences on signaling cascade/s [5]. One of the main mechanisms by which PBMT is achieved is by the generation of energy and the production of reactive oxygen species (ROS) in cells and tissues, particularly in an injured milieu [5]. The pathways sensitive to redox signaling molecules, such as the nuclear factor-κB (NF-κB) transcription factor and mitogen-activated protein kinases (MAPKs), or even growth factors (such as transforming growth factor-β1) are mostly activated by PBMT [6, 7].

Several studies have been carried out in order to investigate the cellular and molecular events that explain the effects of light on organic systems [8–10]. The participation of photoreceptors located on the mitochondrial surface has already been demonstrated in this process [11]. The terminal enzyme of the respiratory chain, cytochrome *c* oxidase (C*c*O), appears to be responsible for the absorption of wavelengths in the region of

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10103-019-02813-5) contains supplementary material, which is available to authorized users.

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red (630 nm) to near-infrared (around 1000 nm) light and possibly also in the region of blue light [11, 12]. On the other hand, opsins and flavoproteins may be responsible for the absorption of wavelengths in the region of blue (around 420 nm) and green light (around 540 nm) [13–15]. The activation of retinal opsins by blue light, for instance, can generate ROS, which are partly responsible for ocular phototoxicity caused by violet and blue light [14]. As such, PBMT may trigger direct or short-term responses represented, among others, by the stimulation of adenosine triphosphate (ATP) synthesis concomitant with transient ROS production [11]. This mechanism, in turn, may be linked to the activation of intracellular cascades that would culminate in indirect or long-term responses such as DNA and RNA synthesis and further regulation of cell functions [16].

A huge number of in vitro studies have demonstrated the benefits of PBMT for cells in culture [1, 17–20]. Biostimulatory responses are generally considered to be in the range of 1.0–10.0 J/cm^2 and the inhibitory effects to be above 25.0 J/cm^2 [21]. Device settings such as power density and exposure time, and other experimental conditions such as the cell type are also important for the biostimulatory action of PBMT [10, 22–24]. Accordingly, the objective of the present study was to carry out a critical review of the literature about the effects of PBMT using wavelengths in the blue, red, and/or near-infrared spectra applied exclusively to keratinocytes cultured in vitro in order to narrow down the most relevant energy densities described in the literature that are able to biomodulate epithelial cells.

Methods

Eligibility criteria

The research question was as follows: What is the best energy density range to be used to produce stimulatory effects on the proliferation, viability, migration and expression of cytokines, growth factors, and NF-κB in keratinocytes cultured in vitro and submitted to PBMT?

Inclusion criteria

Studies in which the LASER/LED was investigated as primary intervention (independent variable), evaluating viability; proliferation; migration; and expression of cytokines, growth factors, and NF-κB, using mammalian keratinocytes cultured in vitro; and LASER/LED operating in blue, red, or near-infrared light and photodynamic therapy (PDT) with positive control of keratinocytes (cells exposed to radiation but not exposed to the photosensitizer) were included.

Exclusion criteria

Studies using only ultraviolet A (UVA) and B (UVB), violet, green, yellow, and orange light; and studies involving confocal laser microscopy, laser scanning cytometry, laser capture microdissection, laser light scattering, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry were excluded. PDT studies without a positive control, studies in which keratinocytes were co-cultured with other cell types, and studies for which only the conference abstract was available were also excluded.

Information sources

Electronic searches without publication date or language restriction were undertaken in February 2018 and later updated in November 2018 in the following databases: PubMed (National Library of Medicine), Web of Science (Thomson Reuters), Scopus (Elsevier), and LILACS (Latin American and Caribbean Center on Health Sciences Information).

Search strategy

The keywords were terms indexed by the Medical Subject Headings (MeSH). The search strategy used for PubMed, Web of Science, Scopus, and LILACS is shown in Table 1. The retrieved references were exported to the EndNote software (Thompson Reuters, New York, NY, USA) and Reference Manager (Mendeley, London, UK). Duplicates were removed upon identification.

Study selection

All titles/abstracts of the references retrieved were assessed independently by two examiners (P.T.R.A. and J.A.A.A.). The inter-examiner kappa score was calculated for the determination of the level of agreement between examiners regarding the assessment of titles/abstracts of 10% of the number of references retrieved ($\kappa = 0.9$). Titles/abstracts that met the eligibility criteria were included in this critical review. When the title/abstract did not contain enough information for a decision about inclusion or exclusion, the full texts were retrieved and evaluated by the same two examiners who applied the same eligibility criteria. Those references that met the eligibility criteria were also included in this critical review. In cases of a divergence of opinion, a third examiner (I.M.A.D.) with expertise in PBMT made the final decision.

Data extraction

The following data were extracted from the included articles: author(s)' name and year of publication, keratinocyte

Table 1 Search strategy for each searched electronic database

Database	Search strategy
PubMed	photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR PBMT OR PBM OR LLLT OR laser OR "light emitting diode" OR "red laser therapy" OR "red laser therapies" OR "infra-red laser therapy" OR "infra-red laser therapies" OR "low intensity laser therapy" OR "low intensity laser therapies" OR "low-intensity laser therapy" OR "low-intensity laser therapies" OR "low level laser therapy" OR "low level laser therapies" OR "low-level laser therapy" OR "low-level laser therapies" OR "low level light therapy" OR "low level light therapies" OR "low-level light therapy" OR "low-level light therapies" OR "low power laser therapy" OR "low power laser therapies" OR "low-power laser therapy" OR "low-power laser therapies" OR "low power laser irradiation" OR "low-power laser irradiation" OR "photobiomodulation therapy" OR "photobiomodulation therapies" OR "laser biostimulation" OR "laser phototherapy" OR "laser phototherapies" AND keratinocyte OR keratocyte
Web of Science	photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR light emitting diode OR red laser therapy OR red laser therapies OR infra-red laser therapy OR infra-red laser therapies OR low intensity laser therapy OR low intensity laser therapies OR low-intensity laser therapy OR low-intensity laser therapies OR low level laser therapy OR low level laser therapies OR low-level laser therapy OR low-level laser therapies OR low level light therapy OR low level light therapies OR low-level light therapy OR low-level light therapies OR low power laser therapy OR low power laser therapies OR low-power laser therapy OR low-power laser therapies OR low power laser irradiation OR low-power laser irradiation OR photobiomodulation therapy OR photobiomodulation therapies OR laser bioestimulation OR laser phototherapy OR laser phototherapies AND keratinocyte OR keratocyte
Scopus	photostimulation OR photoirradiation OR photoactivation OR photobiomodulation OR laser OR "light emitting diode" OR "red laser" OR "low intensity laser" OR "low level laser" OR "low level light" OR "low power laser" OR biostimulation OR phototherapy AND keratinocyte OR keratocyte
LILACS	laser AND keratinocyte OR keratocyte

source used, light device parameters [manufacturer, wavelength in nanometers (nm), operation mode (continuous or pulsed), power in watts or milliwatts (W/mW), power density (W/cm^2 or mW/cm^2), energy density (J/cm^2), total energy in Joules (J) and spot area (cm^2)], treatment specifications (irradiation time (s), irradiation frequency, irradiation performed in contact or at a distance mode); assays performed and replicates; and the main results obtained, including mean and standard deviation/standard error.

The parameters of the LASER/LED devices considered in the present critical review were established according to the recommendations of the World Association for Laser Therapy (WALT) [25]. An accurate report of light parameters defined the study as reproducible and comparable. If necessary, author(s) were contacted to retrieve additional information. In cases in which the author(s) did not provide information about energy density (ED), the calculation was carried out according to the following formula: ED (J/cm^2) = P $(W) \times T$ (seconds) / spot area (cm^2). When the spot area was not provided by the author(s), calculation was performed using the following formula: Area = π (3.14) × r^2.

Data were grouped according to the visible light spectrum (blue, red, or near-infrared) and according to the energy densities used. Thus, arbitrary subdivisions of energy densities were created to compare the studies: 0.1–5.0 J/cm^2, 5.1–10.0 J/cm^2, 10.1–15.0 J/cm^2, and above 15.0 J/cm^2. Studies that fitted into more than one energy density subgroup were allocated to more than one category.

Quality assessment

The quality of the included studies was analyzed by evaluating eight items of the criteria of the Office of Health Assessment and Translation (OHAT) tool [26]. The items were as follows: (1) randomization of treatments, (2) adequate allocation of study groups, (3) experimental conditions of the culture, (4) complete data without any experimental group excluded from the analysis, (5) reliability in the exposure characterization, (6) reliability of the results, (7) statement on the results of all the tests performed, and (8) whether there were no other potential risks to the internal validity.

For each item, the included article was rated as "yes (if the item was acknowledged in the study)" or "no (if the item was not acknowledged in the study)," or "unclear (if it was impossible to state if the item was acknowledged)." For example, studies' missing information about the use of a method to avoid cell over-irradiation and/or for device power checking prior to the experiments were rated as "unclear" and, consequently, with a potential risk of internal validity.

Results

Study selection

A total of 3221 references were identified in all databases searched. After the removal of duplicates, the titles/abstracts of 1997 references were evaluated. Of these 1997 titles/abstracts, 1860 were excluded because they did not meet the criteria and 137 were selected for full text assessment. Fifty-five met the eligibility criteria and were included in this critical review (Supplementary References 1). The flowchart shown in Fig. 1 depicts the search and the selection process.

Irradiation parameters

Supplementary Tables 1, 2, and 3 show the studies grouped according to visible light spectrum, source of keratinocytes, energy densities, assays performed, experimental times and results regarding cell proliferation, viability, migration, expression of cytokines, and growth factors. The results refer to the irradiated groups in relation to the non-irradiated control groups.

Of the 55 studies analyzed, 14 (25.5%) used blue light (Supplementary Table 1), 34 (61.8%) used red light (Supplementary Table 2), and 17 (30.9%) used infrared light (Supplementary Table 3). Wavelengths ranged from 405 nm (blue) to 1064 nm (near-infrared). The variation was 405–488 nm in the blue spectrum, 622–672 nm in the red spectrum, and 780–1064 nm in the infrared spectrum. The energy density (J/cm^2) was the most frequently reported parameter for all wavelengths, ranging from 0.3 to 168.5 J/cm^2 in the blue spectrum, from 0.1 to 271.0 J/cm^2 in the red spectrum, and from 0.12 to 1080 J/cm^2 in the infrared spectrum. Two studies did not report the energy density used and, in this case, we performed the calculation using the formula described above.

Fifty studies (90.9%) did not report the use of optical fibers, 47 (85.4%) did not report the total energy (J) supplied to the cells, and 32 (58.1%) did not report the spot area. Twenty-eight studies (50.9%) did not report the distance between the device tip and the irradiated cells, the operation mode, or the power used (Supplementary Table 1).

Effects on viability and proliferation

Of the 55 studies, 50 (90.9%) evaluated the effects of PBMT on cell viability and proliferation. Of these, 42 (84.0%) evaluated proliferation and viability over short experimental times (a maximum of 48 h after irradiation), seven (14.0%) evaluated the effects of PBMT over long experimental times (more than 48 h), and five (10.0%) did not report the experimental

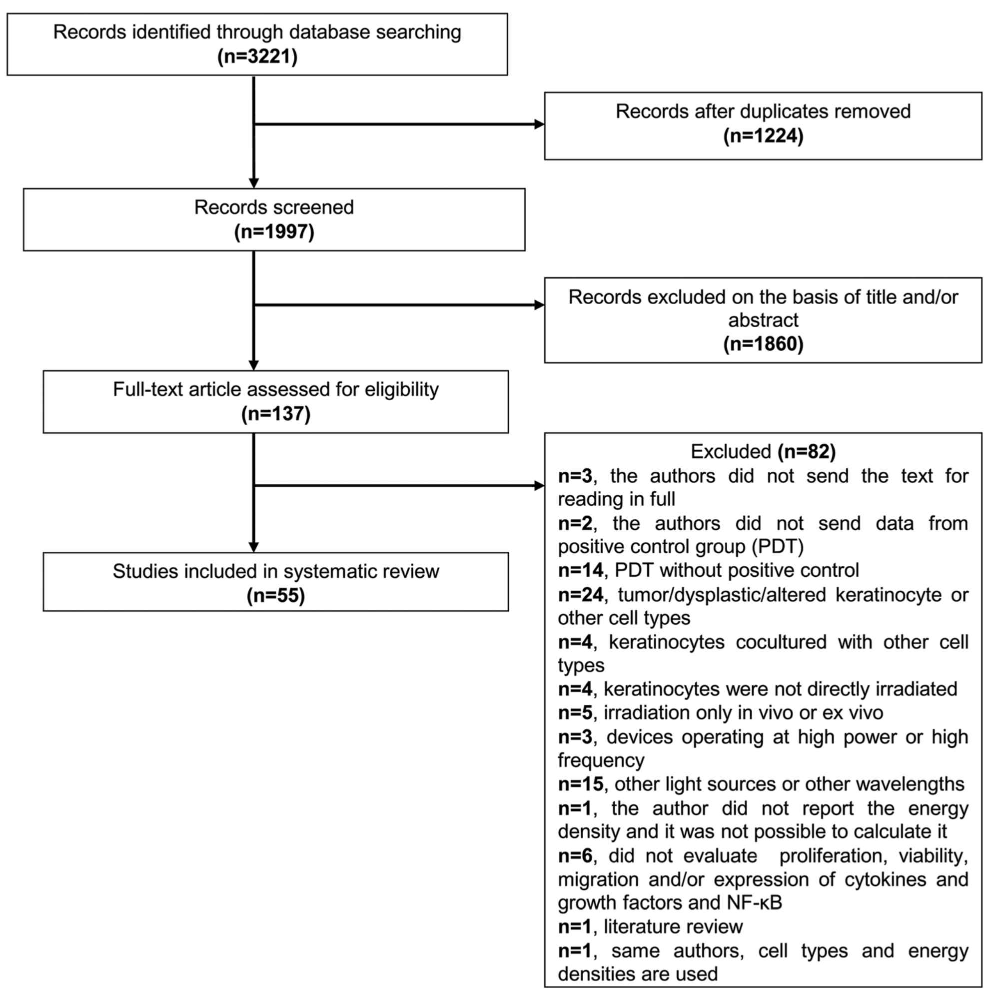


Fig. 1 Flowchart showing the result of the search process

time used. Of the studies using short experimental times, 17 (40.5%) evaluated proliferation and viability over experimental times below 24 h, 31 (73.8%) evaluated the effects of PBMT 24 h after irradiation, and six (14.3%) performed the evaluation 48 h after irradiation. Of the studies using long experimental times, six (85.7%) evaluated the effects of PBMT 72 h after irradiation, and only one (14.3%) study performed the evaluation over experimental times of more than 72 h, particularly on the fifth and eighth day after irradiation. Several studies evaluated the effects of PBMT during more than one experimental time. The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and Trypan Blue test were the assays most frequently used for the assessment of cell viability/proliferation (Supplementary Tables 2, 3, and 4).

Cell viability/proliferation outcomes according to the wavelength and energy density ranges are summarized in Fig. 2. Of the 50 studies assessing the effects of PBMT on cell viability and proliferation, 13 (26.0%) evaluated the effects of blue light. Of these, three studies detected an increase in viability and proliferation in the range of 0.1–5.0 J/cm^2. No cell death was reported at this range. Five studies also reported that this energy density range did not modify viability or proliferation.

The range of 5.1–10.0 J/cm^2 did not modify cell viability/proliferation or cell death. In the range of 10.0–15.0 J/cm^2, only one study reported reduction in cell viability, specifically at 15.0 J/cm^2.

Under doses above 15.0 J/cm^2, most studies ($n = 7$) found no change in viability or proliferation. However, three studies detected cell death at 20.0 and 40.0 J/cm^2; 33.0 at 66.0 and 100.0 J/cm^2; and at 25.0, 45.0, 50.0, and 85.0 J/cm^2 energy density (Supplementary Table 2).

Of the 50 studies that evaluated the effects of PBMT on cell viability and proliferation, 31 (62.0%) evaluated the effects of red light. Stimulatory effects were observed in the range of 0.1–5.0 J/cm^2. Only one study detected a reduction in cell viability at this range, specifically at 3.6 J/cm^2. Most studies ($n = 14$) reported no effects on viability or proliferation.

When doses in the range of 5.1–10.0 J/cm^2 were used, only one article reported increased cell viability at the dose of 6.0 J/cm^2, and only one reported reduction in cell proliferation, in particular at the dose of 10.0 J/cm^2. Nine studies reported no changes in cell proliferation or viability at this range.

Four studies reported no change in cell viability or proliferation in the range of 10.1–15.0 J/cm^2. On the other hand, one study showed an increase in cell viability at 12.0 J/cm^2. There was no cell death in this range. Similar results were obtained at doses greater than 15.0 J/cm^2, with which most studies ($n = 10$) found no change in viability or proliferation. On the other hand, two studies reported an increase in cell viability at the doses of 271.0 J/cm^2 and 30.0 J/cm^2, and two studies reported a reduction in cell viability at the doses of 30.0 J/cm^2 and 112.5 J/cm^2 (Supplementary Table 3).

Sixteen of the 50 studies (32.0%) evaluated the effects of near-infrared light on cell viability and proliferation. An increase was observed in the range of 0.1–5.0 J/cm^2 and did not detect any changes regarding cell viability/proliferation. Two studies reported a reduction in cell viability and proliferation.

An increase in cell proliferation was observed in the range of 5.1–10.0 J/cm^2. In contrast, four other studies that evaluated cell viability at doses within this range did not detect any change.

Only two studies evaluated cell viability using energy doses in the range of 10.1–15.0 J/cm^2, and neither one observed any changes compared to the control group. Eight studies evaluated the effects of PBMT on cell viability/proliferation using doses above 15.0 J/cm^2. Six did not report changes in the irradiated group compared to control; one detected a reduction in cell viability and one detected increased

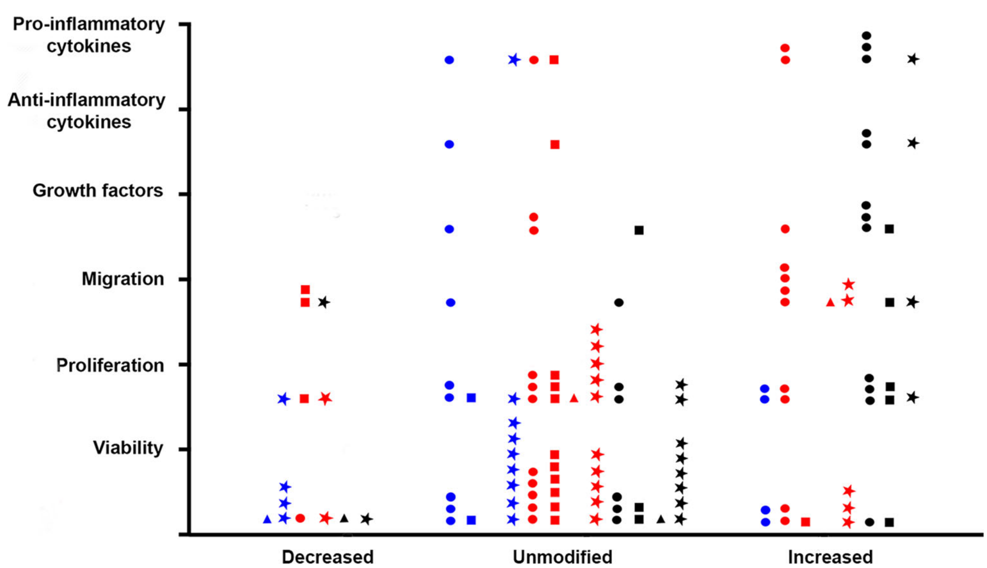


Fig. 2 PBMT effects on cell viability, proliferation, migration, growth factors, and anti-inflammatory and pro-inflammatory cytokines released according to the energy density (J/cm^2) applied. The symbols represent the energy density ranges within the blue (blue), red (red), or near-infrared (black) spectra: circle 0.1 to 5.0 J/cm^2; square 5.1 to 10.0 J/cm^2; triangle 10.1 to 15.0 J/cm^2; and star, above 15.0 J/cm^2

proliferation, in particular at the dose of 32.47 J/cm^2 (Supplementary Table 4).

Effects on migration

Of the 55 studies included, only nine (16.3%) evaluated the effects of PBMT on cell migration. These studies investigated cell migration over short experimental times (48 h after irradiation). Six studies (66.6%) evaluated cell migration over experimental times below 24 h. Nine studies (100%) evaluated the effects of PBMT 24 h after irradiation, and two (22.2%) within 36 and 48 h after irradiation. Some studies evaluated migration over more than one experimental time. All studies evaluated cell migration using the cell scratch assay. These data are reported in Supplementary Tables 2, 3, and 4.

Cell migration outcomes according to the wavelength and energy density ranges are summarized in Fig. 2. Of the nine studies in which the effects of PBMT on cell migration were evaluated, two (22.2%) used blue light. In these studies, the doses of 0.3 J/cm^2 and 2.0 J/cm^2 did not produce changes compared to control. When 30.0 J/cm^2 was used, there was a reduction in migration (Supplementary Table 2).

Six studies (66.6%) evaluated the effects of PBMT on cell migration using red light. In this case, stimulatory effects occurred in the 0.1–5.0 J/cm^2 range, particularly when the cells were irradiated three times with 0.8 J/cm^2, 0.6 J/cm^2, 4.0 J/cm^2, and 0.1, 0.2 and 1.2 J/cm^2. Two studies evaluated cell migration in the range of 5.1–10.0 J/cm^2; one of them detected an increase in migration with 8.0 J/cm^2 and the other reported a reduction of migration with 10.0 J/cm^2. There was also an increase in cell migration in the range of 10.1–15.0 J/cm^2 when using the dose of 12.0 J/cm^2; and in the range > 15.0 J/cm^2, when using 20.0 J/cm^2 (Supplementary Table 3).

Of the nine studies which evaluated the effects of PBMT on cell migration, only two (22.2%) used infrared light. One reported an increase in cell migration at 6.5, 16.23, 32.47, and 48.7 J/cm^2 and the other reported no effects at 1.2 J/cm^2 compared to the non-irradiated controls (Supplementary Table 4).

Effects on the production of cytokines and growth factors

Fifteen of the 55 studies included (27.2%) evaluated the effects of PBMT on the production of cytokines, growth factors, and/or NF-κB. The expression of cytokines and growth factors was evaluated at time points of less than 24 h in five of these (33.3%), 24 h after irradiation in 10 (66.6%), 48 h after irradiation in one (6.6%), and 96 h after irradiation in one. In four (26.6%) studies, the experimental time used was not reported. Some studies evaluated the effects of PBMT during more than one experimental time. The assays performed are described in Supplementary Tables 2, 3, and 4.

The cytokines analyzed were interleukin (IL)-1alpha (IL-1α), IL-1beta (β), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α), transforming growth factor-alpha (TGF-α), TGF-β, interferon-gamma (IFN-γ), the stem cell factor (SCF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). The growth factors studied were heparin-binding EGF-like growth factor (HB-EGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), and epidermal growth factor (EGF). Three studies evaluated NF-κB transcription factor.

Information about the expression of cytokines, growth factors, and other molecules according to the wavelength and energy density used is summarized in Fig. 2. Of the 15 studies in which the effects of PBMT on production of cytokines, growth factors, and NF-κB were evaluated, three (20.0%) used blue light. Energy densities in the range of 0.1–5.0 J/cm^2 did not modify the expression of these molecules, as also observed with the range above 15.0 J/cm^2. On the other hand, one study detected downregulation of NF-κB, TGF-β, and TNF-α with 41.4 J/cm^2 (Supplementary Table 2).

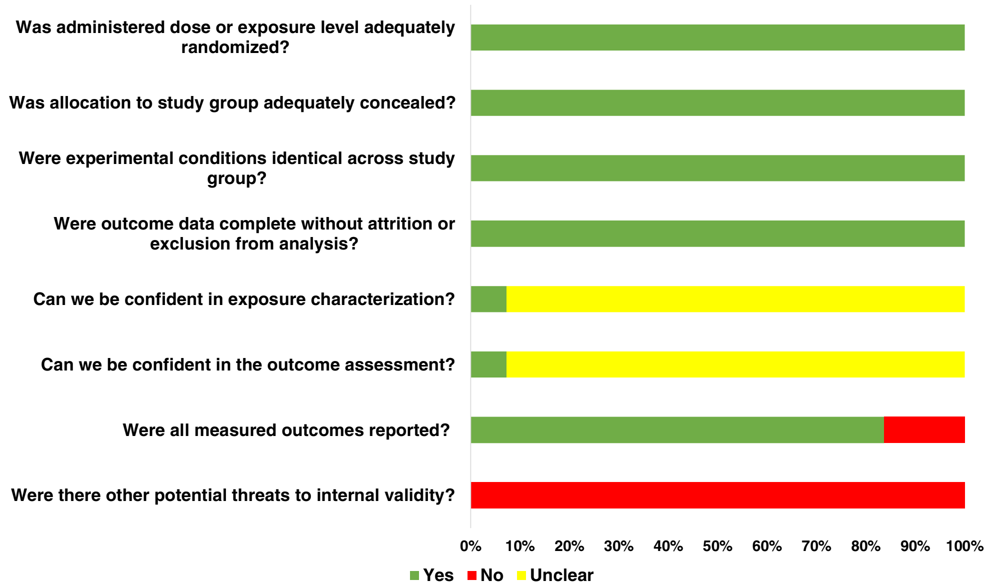
Of the 15 studies in which the effects of PBMT on the production of cytokines, growth factors, and NF-κB were evaluated, six (40.0%) used red light and seven (46.6%) used infrared light. The effects of red and infrared light on the expression of cytokines, growth factors, and NF-κB can be seen in Supplementary Tables 3 and 4, respectively. There was an increase in the production of cytokines and growth factors in the range of 0.1–5.0 J/cm^2. In this range, an increase of IL-1α, IL-6, IL-8, bFGF, NGF, EGF, KGF, VEGF, TGF-α, TGF-β, and TNF-α was reported. One study performed a wound on the cells' monolayer (cell scratch assay) and showed that NF-κB was activated in the irradiated cells placed on the edge of the wound. In the range of 5.1–10.0 J/cm^2, there was no change in the expression of IL-1α, IL-6, TNF-α, or TGF-β with the use of red light or in the expression of bFGF with the use of infrared light. On the other hand, an increased expression of VEGF, TGF-β, IL-1α, IL-6, TNF-α, and TGF-β was reported. No change in NF-κB expression was observed in the range above 15.0 J/cm^2.

Quality assessment

Figure 3 shows the established criteria for the quality and risk of bias of each in vitro study according to the OHAT tool. Overall, we observed that all 55 studies included met the criteria of randomization, adequate allocation of groups, and experimental conditions in a similar manner. On the other hand, nine studies (16.3%) did not show the values of some of the assays performed.

All the 55 studies included herein were considered to have a potential risk of internal validity, since they failed to

Fig. 3 Quality assessment of the included studies



accurately provide the LASER/LED light parameters, or to report a method for over-irradiation prevention, or to inform whether power was checked before the experiments (Supplementary Table 5). Only two (3.6%) studies reported all light parameters. Only eight (14.5%) studies reported the use of methods aiming to avoid over-irradiation of the experimental groups, such as empty wells adjacent to the test wells or the use of black paper. Twenty (36.3%) studies reported power checking of the apparatus prior to the experiments, such as the use of a power meter.

Discussion

The epithelium is an important barrier that protects the body from external aggression. Therefore, restoring its integrity upon injuries is of paramount importance for the maintenance of health [19]. The development of tools that stimulate the viability/survival, proliferation, and migration of the epithelial constituent cells, such as keratinocytes, may have therapeutic benefits. This critical review showed that both cell viability and proliferation were stimulated by PBMT mainly at energy doses within the range of 0.1–5.0 J/cm^2 in any of the three light spectra analyzed (blue, red, and infrared). Cell migration and an increased expression of cytokines were also observed, particularly after red and infrared light irradiation. Wide heterogeneity was found among studies, thus limiting comparison of the results.

The energy range of 1.0–10.0 J/cm^2 reported in the study of Tata and Waynant [21] is the classical therapeutic window for PBMT by promoting biomodulatory effects. Accordingly, the present critical review shows that for epithelial cells, the ideal range may be narrower than the 1.0–10.0 J/cm^2, i.e., around 0.1–5.0 J/cm^2. However, more than the energy density, the biological effects of PBMT may be dependent on the rate at which light is delivered, which is the power density of the light (W/cm^2) [12]. This may explain why some studies using energy densities above 5.0 J/cm^2 also obtained positive results. For instance, the increase in proliferation and viability detected in the range of 5.1–10.0 J/cm^2, when red or infrared light was used, occurred at the doses of 6.0 J/cm^2, 6.5 J/cm^2, and 7.0 J/cm^2 [19, 27, 28], which are barely above the 0.1–5 J/cm^2 range. In contrast, interesting positive results were obtained in the > 15.0 J/cm^2 range, with 271.0 J/cm^2, 30.0 J/cm^2, and 32.47 J/cm^2 [19, 29, 30]. Unfortunately, we could not analyze all the included studies also considering the power density used due to failure in reporting important light parameters in many studies. Moreover, the wide diversity of light setting protocols caused data summary to be highly speculative.

In fact, our results corroborate those based on the Arndt-Schultz curve reported elsewhere [12, 31], in which a bimodal effect of PBMT was observed at relatively close energy densities. We observed that when higher densities (over 10.0 J/cm^2) were tested, cell proliferation and viability were reduced [3, 32–36]. One possible explanation may be related to the sensitive balance between ROS levels and ROS scavengers in cells and tissues before and after PBMT. Accordingly, a moderate increase in ROS can promote cell proliferation and differentiation, whereas excessive amounts of ROS can be toxic to the cells, causing oxidative damage to lipids, proteins, and DNA [29]. In this respect, Gagnon et al. [32] reported that the dose of 10.0 J/cm^2 produced the worst results regarding proliferation and migration, similar to those of the experimental group cultured with 1% fetal bovine serum, i.e., under oxidative stress. Lower doses (0.1 and 0.2 J/cm^2), yielded positive results similar to those for the non-irradiated group cultured in 10% fetal bovine serum, which provided ideal conditions for cell culture growth. As such, the control of ROS generation in PBMT is apparently challenging regarding the induction of beneficial and detrimental outcomes [12].

Considering the effects of PBMT on the expression of cytokines, the analysis must consider the plethora of effects produced by these molecules [37]; e.g., IL-1α, IL-8, and TGF-β play a role in keratinocyte proliferation and migration [38–40]. The growth factor VEGF and the platelet-derived endothelial cell growth factor (PD-ECGF) are involved in angiogenesis, and interleukins (4, 5, and 8) act as chemotactic factors in inflammatory cell recruitment [41]. In the study of Baroni et al. [42], cytokines were evaluated in HaCaT cells infected with *Candida albicans* treated or not with irradiation. The fungus-infected and non-irradiated cells produced high levels of the proinflammatory cytokines IL-8 and TNF-α and low levels of TGF-β compared to the irradiated cells. Irradiation with 2.0 and 4.0 J/cm^2 caused a reduction of proinflammatory cytokines and an increase of TGF-β in infected cells.

It should be noticed that most energy densities and wavelengths tested in the studies, regardless of the assays performed, yielded unmodified outcomes. This observation is particularly evident in the viability and proliferation assays, since they are more frequently used as a basic parameter of cytotoxicity in the in vitro studies. A common feature of these studies is the lack of reproduction of in vitro conditions that mimic an injured milieu before testing PBMT. Apparently, the redox signaling that occurs in PBMT can have opposite effects on healthy and stressed cells [12]. As such, challenging cells chemically, reducing fetal bovine serum concentration (mimicking oxidative stress), decreasing pH, simulating hypoxia, or wounding may represent a struggle for cell survival where PBMT may represent a benefit [39, 43–45].

An important issue observed in data condensation was variation in treatment protocols and cell culture conditions, which may have resulted in different outcomes even when using the same light parameters [44]. Moreover, cell viability, proliferation, migration, and cytokine and growth factor expression patterns can be influenced in a dynamic time-varying manner, thus limiting data grouping and analysis. Indeed, the vast majority of the reviewed studies did not provide enough information either about the exposure protocols or about the outcomes assessed. Carefulness in providing full device setting guarantees reproducible studies and external validity. In fact, the OHAT tool was developed in 2014 to assess potential bias for in vitro and mechanistic studies [26]. Accordingly, the OHAT is a checklist that may help researchers to be aware of the internal and external validity of their experimental design and/or data description. Thus, we strongly recommend that future experimental studies follow the OHAT guidelines.

Conclusions

In summary, the present critical review demonstrated the heterogeneity of PBMT effects on keratinocytes cultured in vitro, which may be due to the different protocols and light parameters used. Most tested energy densities produced unmodified results regardless of the wavelength applied. In contrast, an increase of cell viability, proliferation, migration, and expression of cytokines and growth factors was observed when red or near-infrared light was used at doses from 0.1 to 5.0 J/cm^2. The heterogeneity of the data did not allow us to define a PBMT protocol that would induce beneficial effects on keratinocytes.

Acknowledgments Mrs. E. Greene provided English editing of the manuscript.

Funding information This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). T.A.S. and R.A.M. are CNPq research fellows. P.T.R.A. and J.A.A.A. are the recipients of fellowships.

Compliance with ethical standards

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

Ethical approval Ethical approval is not applicable in this study.

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