THE ROLE OF LIGHT EMITTING DIODE IN WOUND HEALING: A SYSTEMATIC REVIEW OF EXPERIMENTAL STUDIES

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Abstract

Background: Wounds represent a growing global issue demanding increased attention. To expedite wound healing, technologies are under development, and Light Emitting Diode (LED) devices of varying wavelengths are being explored for their stimulating influence on the healing process. This article presents a systematic literature review aiming to compile, organize, and analyze the impacts of LED devices on wound healing. Methods: This review is registered on the PROSPERO platform [CRD42023403870]. Two blinded authors conducted searches in the Pubmed, Web of Science, Scopus, Embase, and ScienceDirect databases. In vitro and in vivo experimental studies assessing LED utilization in the wound healing process were included. Results: The search yielded 1010 studies, of which 27 were included in the review. It was identified that LED stimulates different healing pathways, promoting enhanced cell proliferation and migration, angiogenesis stimulation, increased collagen deposition and modulation of the inflammatory response. Conclusions: Thus, it can be concluded that the LED stimulates cellular and molecular processes contingent on the utilized parameters. The effects depend on the standards used. Cell migration and proliferation were better influenced by green and red LED. The extracellular matrix components and angiogenesis were regulated by all wavelength and the modulation of inflammation was mediated by green, red and infrared LEDs.
FUNDING
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST DISCLOSURE
The authors declare no conflict of interest.

ABSTRACT
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Conclusions: Thus, it can be concluded that the LED stimulates cellular and molecular processes contingent on the utilized parameters. The effects depend on the standards used. Cell migration and proliferation were better influenced by green and red LED. The extracellular matrix components and angiogenesis were regulated by all wavelength and the modulation of inflammation was mediated by green, red and infrared LEDs.

KEYWORDS: LED; Phototherapy; Low-Level Light Therapy; Wound healing; In Vitro Techniques; Animal Experimentation.

SIGNIFICANCE STATEMENT
Several author have studied the different mechanisms of action that justify the effect of LED on healing. However, no analysis was carried out combining these results. This systematic review, analyzed and discussed the effects of LED based on results of different studies, with analysis of the action of wavelengths. LED acts in all phases of wound healing through different mechanisms. Cell migration, cell proliferation and inflammation appear to be most influenced by green and red wavelengths. The extracellular matrix and angiogenesis can be influenced by all wavelengths. The results of this study may encourage the construction of new devices with wavelengths and enhance healing effects.

INTRODUCTION
Wound healing is characterized as several physiological processes whose purpose is to repair an injury. These steps have been classified into: homeostasis, inflammation, proliferation and tissue remodeling (Arribas-López, Zand, Ojo, Snowden, & Kochhar, 2021; Magni et al., 2022). The physiological regulation of cutaneous wound healing depends on many types of immune cells and mediators, which interact in a highly sophisticated temporal sequence (Wang, Huang, Horng, Yeh, & Chen, 2018). Although traditional therapies such as ointments, dressings and bioactive compounds are currently in practice, these strategies are insufficient to contribute to the full stages of healing (Lee et al., 2022).

Studies in the literature have reported the development of new increasingly efficient approaches to tissue repair. Among current approaches are the use of photobiomodulation, promoted by Light Emitting Diode devices (LED) and the use of Light Amplification by Stimulated Emission of Radiation (LASER), which has shown good results and attracted the attention of the scientific community (Chaves, Araújo, Piancastelli, &
Pinotti, 2014; Silveira et al., 2016). However, the LED has advantages that include low cost, portable device, home usability, with the ability to irradiate a large tissue area at once (Martignago et al., 2020).

Phototherapy using this type of device has shown promising biological effects. The initial interaction between the light radiated by the LED device and the cellular photoreceptors is followed by the activation of multiple molecular mediators. These eventually lead to major changes in gene expression, cell signaling, cell metabolism, and cytokine secretion (Heiskanen & Hamblin, 2019) triggering intracellular biochemical reactions, such as an increase in ATP production, modulation of oxidative stress, induction of transcription factors, changes in collagen synthesis, angiogenesis stimulation, and increased blood flow (Gomes et al., 2016; Sorbellini, Rucco, & Rinaldi, 2018).

Light absorption is the main mechanism that allows LED to modulate cellular and molecular processes associated with healing; thus, the way LED light interacts with biological tissue depends on some properties and parameters of the optical device, mainly wavelength (nm), dose (J/cm²), power (mW/cm²), time (t), as well as the optical properties of the tissue (Chaves et al., 2014; de Alencar Fernandes Neto, Nonaka, & de Vasconcelos Catão, 2019; Gomes et al., 2016; Martignago et al., 2020; Sorbellini et al., 2018).

Depending on the composition of the device, different wavelengths can be generated, and LED systems can provide light in continuous or pulsed mode. The wavelength range can vary from 400 to 1200 nm and longer wavelengths penetrate deeper into the tissue. Different cells and tissues absorb these wavelengths of light, which is closely related to the penetration that the wavelength must achieve, and these devices emit photons that reach blue light (400-470 nm), green light (500-565 nm), red and infrared (600-1000 nm) (de Alencar Fernandes Neto et al., 2019; Sorbellini et al., 2018; H. Zhang et al., 2018).

The diverse effects of LED therapy, contingent upon the interacting cell type and the wavelength employed, render its application in wound healing a formidable challenge. Studies elucidating the mechanisms of action of this therapy can facilitate the selection of optimal parameters and interventions. Consequently, this literature review aimed to aggregate, organize, and summarize the following aspects: I - The biological effects that the use of LED has in vitro and/or in vivo in the wound healing process; II - The predominant LED light parameters, including wavelength and dosage, that are employed in wound treatment; III - A comprehensive elucidation of the cellular-level mechanisms of action exerted by these LED devices to expedite the healing process.

MATERIALS & METHODS

This systematic review was described according to the Recommendation Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA) (Page et al., 2021) and the protocol registered in the International Prospective Register of Systematic Reviews (PROSPERO) [CRD42023403870].

Search strategy

The search was carried out in five databases: Pubmed, Web of Science, Scopus, Embase and Science Direct. The last search was performed in February 2024. Controlled and uncontrolled terms were used: Wound, Wound healing, Wounds and injuries, Light emitting diode, Red LED, Infrared LED and Blue LED. The terms were combined with Boolean operators to compose the search strategy. The strategy used for each database is shown in Figure 1.

Criteria for Selecting Studies

In vitro and in vivo experimental studies that aimed to evaluate the use of LED in the wound healing process were included. There was no restriction regarding the year of publication or language. The following exclusion criteria were adopted: use of LED associated with other treatments, burns, injuries closed through sutures, studies with oral tissue, veterinary studies, and works published in conferences.

Selection of Studies

The studies were analyzed in two stages, according to the eligibility criteria. First, titles and abstracts were
read. The articles selected at this stage underwent a complete reading. The process was performed by two independent authors, and discrepancies were resolved through inter-author discussions and consultation with a third author.

Data Extraction

A data extraction form was prepared by the authors. Data were collected according to the study design regarding sample characterization, information related to the injury induction model, *in vitro* assays, healing rate, results of histological analyses, and DNA and RNA analyses.

Quality Assessment of the Studies

*In vitro* studies were assessed using the methodology employed by KULKARNI et al. (Kulkarni, Meer, & George, 2020), which consists of 8 criteria. Each criterion was assessed with a YES or NO response. The articles were classified as high-risk when more than 5 criteria were evaluated as no, medium-risk with 5 to 2 negative criteria, and low-risk 1 to 0 criteria evaluated as no. For the evaluation of *in vivo* studies, SYRCLE’s Rob tool (Hooijmans et al., 2014) was used. This tool contains 10 items individually evaluated for the presence of bias such as yes, no or unclear.

Data Analysis

Heterogeneity in the data and the absence of a description of absolute values in the studies were identified. Thus, it was not possible to perform a meta-analysis. Therefore, a descriptive analysis of the data was performed.

RESULTS

The search yielded 1010 studies. After eliminating duplicates and reviewing the titles and abstracts, 108 studies were subjected to full-text examination, resulting in the inclusion of 28 papers in the review. The selection process is shown in Figure 2.

Among the experimental studies incorporated into the review, 14 adopted an *in vitro* experimental design involving cell cultures, 13 employed *in vivo* preclinical assays with mice, and 2 studies utilized both methodologies. Table 1 summarizes the main characteristics and results of the studies.

LED characterization

The wavelength referring to red light was the most applied, which was used by thirteen studies (Cha et al., 2024; Choi et al., 2019; Corazza et al., 2007; Dall Agnol et al., 2009; Gomes et al., 2016; Hsieh, 2013; Huang et al., 2016; Jeon et al., 2018; Kim et al., 2015; Komine et al., 2010; Mo et al., 2019; Paraguassu et al., 2014; Song et al., 2003) and eleven other studies used this to compare with other wavelengths (Adamskaya et al., 2011; Chellini et al., 2020; Cheon et al., 2013; de Sousa et al., 2010; Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017; Teuschl et al., 2015; Vinck et al., 2003, 2005; Zhao et al., 2022). Only seven authors investigated the effects of infrared LED (Chellini et al., 2020; de Sousa et al., 2011, 2014, 2010; Vinck et al., 2003, 2005; Zhao et al., 2022). Nine studies verified the effects of blue LED (Adamskaya et al., 2011; Chellini et al., 2020; Cheon et al., 2013; de Sousa et al., 2010; Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017; Rossi et al., 2021; Teuschl et al., 2015) and seven of green LED (Cheon et al., 2013; de Sousa et al., 2010; Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017; Vinck et al., 2003, 2005). Only one study used the combination of wavelengths in a cluster (Leite et al., 2014). Two studies verified the effects of innovative LED-based devices for healing (Jeon et al., 2018; Phan et al., 2021).

Cellular and molecular mechanisms

*Cell viability and proliferation*

Twelve studies evaluated cell viability and proliferation through assays of the MTT, CCK-8, Sulforhodamine B-based assay, Syto16/PI or MTS (Chellini et al., 2020; Choi et al., 2019; Huang et al., 2016; Jeon et al., 2018; Kim et al., 2015; Phan et al., 2021; Rohringer et al., 2017; Rossi et al., 2021; Teuschl et al., 2015; Vinck et al., 2005; Zhao et al., 2022). Nine studies verified the effects of blue LED (Adamskaya et al., 2011; Chellini et al., 2020; Cheon et al., 2013; de Sousa et al., 2010; Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017; Rossi et al., 2021; Teuschl et al., 2015) and seven of green LED (Cheon et al., 2013; de Sousa et al., 2010; Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017; Vinck et al., 2003, 2005). Only one study used the combination of wavelengths in a cluster (Leite et al., 2014). Two studies verified the effects of innovative LED-based devices for healing (Jeon et al., 2018; Phan et al., 2021).
et al., 2005, 2003; Zhao et al., 2022). The MTT was the most frequent test, used by ten studies. LED did not present a cytotoxic effect, and depending on the wavelength and dose, it increased cell proliferation. The greatest stimulus to proliferation was related to lower doses and wavelengths between 516 and 950 nm. Only one study related blue light (420 nm) to increased cell proliferation of human fibroblasts (Rossi et al., 2021). Three studies reported reduced proliferation or no influence of blue light on the viability of endothelial cells, human and murine fibroblasts, myoblasts and keratinocytes (Chellini et al., 2020; Rohringer et al., 2017; Teuschl et al., 2015).

The green LED, when compared to the red LED, can have the same effect or have a superior result in cell viability tests (Phan et al., 2021; Rohringer et al., 2017; Vinck et al., 2003). On the other hand, when comparing the red LED to the infrared spectrum, the red LED showed better results (Phan et al., 2021). Regarding the parameter used, increasing the dose used may reduce cell metabolism or have an effect like not applying the treatment (Phan et al., 2021; Rossi et al., 2021; Zhao et al., 2022). According to a study by Zhao et al., carried out with LED 628 nm, doses above 4 J did not influence cell proliferation (Zhao et al., 2022).

**Cell migration**

Nine studies evaluated the effects of LED on cell migration through the scratch wound healing assay, and all results demonstrated an increase in the rate of cell migration in groups treated with LED (Fushimi et al., 2012; Huang et al., 2016; Jeon et al., 2018; Kim et al., 2015; Phan et al., 2021; Rohringer et al., 2017; Rossi et al., 2021; Teuschl et al., 2015; Zhao et al., 2022). The authors verified the action of the LED with a wavelength of 420 to 810 nm and the green LED showed better results (Fushimi et al., 2012; Phan et al., 2021; Rohringer et al., 2017). However, this result may depend on when the migration is evaluated. After four days, Rohringer et al. demonstrated a greater effect of the red LED compared to the green LED in the 3d scratch assay (Rohringer et al., 2017). The wavelengths related to the blue spectrum had less effect on cell migration (Phan et al., 2021; Rohringer et al., 2017; Teuschl et al., 2015).

The studies verified the influence of the chosen dose on cell migration. It was identified that the lowest doses presented better results. Huang et al. reported increased migration with LED 640 nm at a dose of 12 J/cm² compared to the use of 24 J/cm² (Huang et al., 2016). This same author performed a cell motility test and identified an increase in cell trajectory of 41.9% in applying 12 J/cm² and 18.3% with 24 J/cm². The study by Kim et al. obtained better results with LED 660 applied at 5 and 10 minutes when compared to 20 minutes (Kim et al., 2015).

**Gene expression**

Numerous in vitro and in vivo studies employing LEDs at varying wavelengths have demonstrated therapeutic properties associated with the promotion of the healing process. Among the studies evaluated, the majority utilized LEDs emitting red wavelengths. These studies observed modulation in the expression of several proteins and genes, including an upregulation in the expression of vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), metalloproteinase-2 (MMP-2), metalloproteinase-9 (MMP-9), heat shock protein-27 (HSP27), heat shock protein-60 (HSP60), hepatocyte growth factor (HGF), chemokine-16 (CXCL16), hypoxia-inducible factor 1-alpha (HIF-1α), keratinocyte growth factor (KGF), leptin, interleukin-8 (IL-8), transforming growth factor-α (TGF-α), Dnmt3a, cytokeratin-10 (Krt-10), cytokeratin-17 (Krt-17), platelet-derived growth factor (PDGF); proliferating cell nuclear antigen (PCNA) (Adamskaya et al., 2011; Chellini et al., 2020; Cheon et al., 2013; Choi et al., 2019; de Sousa et al., 2011; Fushimi et al., 2012; Huang et al., 2016; Komine et al., 2010; Rohringer et al., 2017; Song et al., 2003; Zhao et al., 2022). On the other hand, there was a decrease in tissue metalloproteinase inhibitor 1 e 2 (TIMP-1 / TIMP-2), cyclooxygenase-2 (COX-2) e cytokeratin-1 (Krt-1) (Adamskaya et al., 2011; Chellini et al., 2020; Tatmatsu-Rocha et al., 2018).

As for the studies using blue LEDs, a modulation in the overexpression of tissue inhibitor of metalloproteinase 1 and 2 (TIMP-1, TIMP-2), dipeptidylpeptidase-IV (DPP-IV), neuregulin1-b1 (NRG1-b1), placental growth factor (PIGF), HGF e KGF (Chellini et al., 2020; Fushimi et al., 2012; Rohringer et al., 2017) and protein reduction MMP-2 e MMP-9 (Chellini et al., 2020). Additionally, green LEDs also modulate some proteins
as demonstrated by an increase in Neuregulin 1 (NRG1-b1), HGF, serpin F1, KGF, IL-8, Leptin e VEGF (Fushimi et al., 2012; Rohringer et al., 2017). The infrared LED positively modulates expression of VEG, transformative growth factor- β (TGF-β) (Zhao et al., 2022), TIMP-1, TIMP-2 (Zhao et al., 2022) and a decrease in MMP-2 e MMP-9 (Chellini et al., 2020).

in vivo wound healing

Macroscopic applications of LED in mice

Closure of the lesion area was documented in seven studies (Adamskaya et al., 2011; Cha et al., 2024; Dall Agnol et al., 2009; Gomes et al., 2016; Leite et al., 2014; Mo et al., 2019; Paraguassú et al., 2014). Among these, five studies investigated the impact of red LEDs (629 to 640 nm), one study compared red LEDs with blue LEDs, and one study utilized a cluster with both red and infrared light (660 and 890 nm). In all these studies, a reduction in the lesion area was observed. It is worth noting that while a higher dosage may influence a more rapid healing rate, only one study identified this correlation (Gomes et al., 2016). Additionally, one study demonstrated a greater reduction in the lesion area when blue LEDs were employed (Adamskaya et al., 2011).

The influence of the use of LED to heal lesions in the presence of comorbidities was verified by three authors (Dall Agnol et al., 2009; Leite et al., 2014; Paraguassú et al., 2014). LED accelerates wound healing, when compared to control, in animals with Diabetes Mellitus. Area reduction was similar to that found after LASER application (Dall Agnol et al., 2009). In malnourished animals, the use of LED can result in healing rates similar to those of animals with good nutrition (NOURISHED) (Leite et al., 2014). In animals with hypothyroidism, the utilization of LED therapy did not yield a significant impact on the healing rate (Paraguassú et al., 2014).

Microscopic analysis

Histological analysis after application of LED 635 to 850 nm demonstrated an increase in inflammatory cells, blood vessels, fibroblasts and collagen (Cha et al., 2024; Corazza et al., 2007; Dall Agnol et al., 2009; Mo et al., 2019). The use of LED can lead to a rapid increase in inflammatory cells in the early and middle stages of healing. On the third to seventh day after treatment with LED, an increase in neutrophils is identified, this does not occur after evaluation of 7 to 10 days (Mo et al., 2019). The reduction of the LED-mediated inflammatory phase can occur in the intermediate to late phase of healing (Dall Agnol et al., 2009).

The LED favors the increase in the number of blood vessels (Corazza et al., 2007; Dall Agnol et al., 2009; Mo et al., 2019). Wavelengths from 630 to 660 nm increase in the volumetric density of the vessels. The increase of vessels may occur within 10 days after treatment (Mo et al., 2019). The proliferation of fibroblasts is another aspect influenced by the application of LED therapy. The studies demonstrated an increase in this component in groups treated with LED 530 e 700nm when compared to controls (Dall Agnol et al., 2009; de Sousa et al., 2010; Mo et al., 2019).

Collagen deposition may be related to the use of LED depending on the wavelength used (Cha et al., 2024; Hsieh, 2013; Mo et al., 2019). The deposition of immature collagen can be identified on the third day after irradiation with LED 630 nm and on the tenth day the presence of mature collagen was verified in most treated tissues when compared to the control. Mo et al. (Mo et al., 2019) did not verify the effect of LED 850 nm in the analysis of the presence of collagen performed on the fifth day.

Quality Assessment of the Studies

Sixteen studies employed in vitro methodologies, with fifteen of them classified as having a medium risk, and one was rated as low risk (Kim et al., 2015). The criteria that received the most negative evaluations included cell passage, the number of losses, and cell handling between facilities.

Thirteen articles conducted in vivo assay and the results are summarized in Figure 2. With the quality assessment, it was possible to observe that criteria like allocation generation and concealment, random
housing, blinding of outcome assessment, and blinding of experimentalists were not detailed by the authors, and for this reason, were classified as “unclear”. None of the items were classified as high risk.

**DISCUSSION**

This review sought to elucidate the primary mechanisms associated with photobiomodulation using LED in the context of wound healing. The analysis revealed that LED therapy facilitates enhanced cell proliferation and migration, modulation of the inflammatory response, heightened fibroblast activity, increased collagen deposition, stimulation of angiogenesis, and reduction of the lesion area. These effects are mediated by the positive modulation of various cytokines and proteins. It is noteworthy that each phase of the healing process relies on specific molecular and cellular mechanisms to facilitate its progression (Fig. 3).

Four phases are known to be involved in wound healing: homeostasis, inflammatory, proliferative and remodeling and maturation. These can occur overlapping and sequentially (W. Zhang, Zhang, Cui, Zhao, & Lei, 2023). Immediately after injury, there is a phase of homeostasis marked by vasoconstriction and activation of the coagulation cascade. The inflammatory phase occurs through the migration of macrophages and lymphocytes (Dam et al., 2023). The increase in the number of cells such as fibroblasts, keratinocytes and endothelial cells followed by apoptosis and cell production represent the proliferative and remodeling phases, respectively (Li et al., 2022)(Fig. 4).

The occurrence of each phase is vital for the occurrence of the others since there is migration of specific cells that promote the release of growth factors and important cytokines to signal the healing cascade (W. Zhang et al., 2023). However, extrinsic or intrinsic factors can compromise the progression of each phase, usually remaining in the inflammatory phase, known as the chronic healing process (Mosca, Ong, Albasha, Bass, & Arany, 2019).

Approaches currently proposed to accelerate the healing process generally act in only one phase (Li et al., 2022). The present review demonstrates that LED is a therapy capable of acting in different stages, since it promotes the stimulation of molecules and cells present in all stages. The red LED has been extensively researched and is closely associated with performance across in vitro studies, as well as all phases of in vivo assays involving macroscopic and microscopic analyses (Choi et al., 2019; Huang et al., 2016; Zhao et al., 2022). The green LED showed a smaller number of studies; however, this may have similar or greater effects to those found after irradiation with the red LED (Jeon et al., 2018; Phan et al., 2021).

In the study by Phan and collaborators, a higher rate of proliferation and migration of L929 cells was identified in the group with green LED irradiation when compared to blue and red LED (Phan et al., 2021). Rohringer and collaborators demonstrated increased proliferation of Human umbilical vein endothelial cells (2D co-cultures) with green (146 %) and red (144 %) LEDs. This result may be related to the proximity between the wavelengths of the red and green LED (Rohringer et al., 2017). However, it is known that the green light band has a shorter wavelength, which implies a smaller photon displacement distance (Tatmatsu-Rocha et al., 2018). Thus, it is necessary to carry out studies on the healing of human skin, given the greater complexity and depth of the tissue.

Conversely, the blue LED exhibited the weakest correlation with in vitro migration and proliferation assays in wound healing. However, in vivo studies have demonstrated a reduction in the lesion area following irradiation with this wavelength (Adamskaya et al., 2011; Cheon et al., 2013). This apparent contradiction could be attributed to the distinct action mechanism of the blue LED, which appears to be more closely associated with the upregulation of cytokines involved in angiogenesis, collagen deposition, and cell differentiation (Dungel et al., 2008; Rohringer et al., 2017). This sets it apart from the other LEDs studied, which demonstrated a higher expression of cytokines linked to fibroblast proliferation and migration (Song et al., 2003; Tatmatsu-Rocha et al., 2018).

Considering the greater complexity of in vivo healing models, other mechanisms may be involved in the healing effects of blue LED. According to a study conducted by Dungel and colleagues, the blue LED, when compared to red and green LEDs, exhibited greater efficacy in releasing mediators associated with nitric
oxide (NO), thereby contributing to the recovery of experimentally inhibited mitochondria (Dungel et al., 2008). A reduction in NO levels is one of the mechanisms associated with delayed wound healing. NO is conducive to the healing process due to its significant role in vascular homeostasis, inflammation regulation, and its antimicrobial properties (Sivaraj et al., 2023).

The present review demonstrated that different wavelengths could modulate the expression of several proteins and genes. The wavelengths corresponding to blue and infrared light appear to be related to angiogenesis and the secretion of substances from the extracellular matrix. Green LED can modulate anti and pro-angiogenic factors, and act on the re-epithelialization and synthesis of EM substances. Red LED is related to the modulation of factors related to angiogenesis, re-epithelialization, and extracellular matrix remodeling, inducing the production of nitric oxide and increasing the expression of adhesion proteins.

Although LEDs with different wavelengths have some similar functions, they may have different activation pathways and expression levels. Thus, the development of devices containing different wavelengths could enhance the effects of this therapy. In this review, only one study associated the use of two wavelengths (red and infrared) (Leite et al., 2014). However, studies are needed to understand the effect of the interaction between the other wavelengths and in which phase of the healing process this junction could be better. A smaller number of studies have verified the effects of infrared LED, so experimental research is needed to better verify the use of this wavelength for wound healing.

In conclusion, the use of LED promotes healing by stimulating mediators involved in all phases. There is modulation of inflammation, stimulation of cell proliferation, cell migration and angiogenesis. The effects depend on the patterns used. Green, red and infrared LEDs influenced the modulation of inflammation mediated by the activation of IL-8, TGF and COX-2. The construction of the extracellular matrix components was positively regulated in all LEDs used and even though it did not depend on a specific wavelength, it was better influenced by infrared and blue light, confirmed by the positive regulation of MMP (2 and 9) and the negative regulation of TIMP (1 and 2). Cell migration and proliferation appear to be more dependent on the applied wavelength, with green and red light demonstrating better results. Angiogenesis, another factor that is independent of the light and wavelength used, can be influenced through different mechanisms, such as the activation of VEGF and leptin by green and red LED and the positive regulation of HGF by blue LED (Fig. 5).

Open issues
This systematic review did not discuss the results according to the assessment of the risk of bias in the studies. It was identified that the in vitro and in vivo articles did not encounter the same criteria present in the tools. Thus, an assessment of the reliability of the results would be unfeasible. It is worth discussing the methodology of experimental studies, are the tools used to assess the risk of bias poorly adapted to this reality, or is there a lack of methodological rigor in carrying out these studies?

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References


**Hosted file**


**Figure 1** - Search strategy according to databases.
Figure 2 - Selection of studies included in the review.
Figure 3 - Mechanisms of photobiomodulation using LED (own construction).

Figure 4 - LED performance in the inflammation phases (own construction).
Figure 5 - Influence of different types of LEDs on the healing process (own construction).

Table 1 - The baseline characteristics and the main results of included studies.

<table>
<thead>
<tr>
<th>References</th>
<th>Studied model</th>
<th>Light</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>(Gomes et al., 2016)</td>
<td><em>in vivo</em>: Mouse</td>
<td>604 nm; 0.8, 1.6 J/cm²;</td>
<td>LED induced a significant reduction in the wound area and the dose of 1.6 J/cm² was related to increased expression of the Dnmt3a gene.</td>
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<td>170 or 340 seconds; 7 days</td>
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<td>(Zhao et al., 2022)</td>
<td><em>in vitro</em>: Mouse fibroblast (L929) <em>in vivo</em>: Mouse</td>
<td>630 or 810 nm; 1, 5 or 10 mW/cm², 100, 200, 500, 1000 or 2000 seconds; 1 or 12 days</td>
<td>The LEDs in 630 and 810 nm induced proliferation and accelerated the migration process <em>in vitro</em>. <em>In vivo</em>, LEDs promoted faster healing and increased VEGF and TGF expression.</td>
</tr>
<tr>
<td>(Phan et al., 2021)</td>
<td><em>in vitro</em>: Human fibroblast cell (L929)</td>
<td>470, 530 or 633 nm; 1.5, 3.0 or 4.5 J/cm²; 5, 10, 15 or 20 seconds/1 day</td>
<td>The use of LED accelerated cell migration and proliferation, with better results with green light.</td>
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<td>(Rossi et al., 2021)</td>
<td><em>in vitro</em>: Human keratinocytes (HaCat) and fibroblast (primary)</td>
<td>420 nm; 3.43, 6.87, 13.7, 20.6, 30.9 or 41.2 J/cm²; 1.2 mW/cm²; 5 or 60 seconds; 1 day</td>
<td>Higher doses reduced metabolism and cell proliferation. In co-cultures, the dose of 20.6 J/cm² stimulated migration at 48 and 72h.</td>
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<tr>
<td>Study</td>
<td>Model</td>
<td>Wavelength (nm)</td>
<td>Power (J/cm², mW/cm²)</td>
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<tr>
<td>(Chellini, Tani, Zecchi-Orlandini, Giannelli, &amp; Sassoli, 2020)</td>
<td><em>in vitro</em>: Human skin fibroblasts (HDF-alfa) and mouse fibroblasts (NIH/3T3)</td>
<td>405, 635 or 808 nm; 0.4 J/cm²; 13 mW/cm²; 30 seconds; 1 day</td>
<td>None of the LEDs showed cytotoxicity. Red LED is related to cell proliferation and remodeling of the extracellular. Blue and infrared LED stimulate the synthesis of type 1 collagen.</td>
</tr>
<tr>
<td>(Choi et al., 2019)</td>
<td><em>in vitro</em>: Human skin fibroblasts</td>
<td>660 nm; 50 mW/cm²; 15, 30 or 60 seconds; 1 day</td>
<td>The LED increased cell activity and expression of HSP27, HSP60, HSP70 and HSP90. The red LED accelerated healing with an increase in the expression of IL-1β and IL-6 in the initial stage of the wound and a decrease in the level of TNF-α in all phases of healing when compared to the non-irradiated group.</td>
</tr>
<tr>
<td>(Mo, Chung, &amp; Ahn, 2019)</td>
<td><em>in vivo</em>: Mouse</td>
<td>630 nm; 6 J/cm²; 5 mW/cm²; 1200 seconds; 14 days</td>
<td>No cytotoxicity effect was observed and the wavelength of 650 and 670 nm were the most effective for cell migration.</td>
</tr>
<tr>
<td>(Jeon et al., 2018)</td>
<td><em>in vitro</em>: Human Fibroblast</td>
<td>630, 650, 670, 690 nm; 3, 6 or 9 J/cm²; 10, 20, 30 seconds; 1 day</td>
<td>Red and green LEDs stimulated cell migration and proliferation in the studied models. Pro-angiogenic factors were upregulated after LLLT. Anti-angiogenic factor serpin F1 was upregulated after green LED.</td>
</tr>
<tr>
<td>(Rohringer et al., 2017)</td>
<td><em>in vitro</em>: Human umbilical vein endothelial cells (HUVEC)</td>
<td>475, 516 or 635 nm; 24 J/cm²; 40 mW/cm²; 10 seconds; 1 or 7 days</td>
<td>LED increases motility, migration, HIF-1α protein with induction of F-actin reorganization.</td>
</tr>
<tr>
<td>(Huang, Qian, Sun, &amp; Cheng, 2016)</td>
<td><em>in vitro</em>: Human keratinocyte cells (primary)</td>
<td>640 nm; 12 or 24 J/cm²; 30 mW/cm²; 6.40 seconds; 1 day</td>
<td></td>
</tr>
<tr>
<td>Study &amp; Authors</td>
<td>Methodology</td>
<td>Organisms</td>
<td>Wavelength (nm)</td>
</tr>
<tr>
<td>----------------</td>
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</tr>
<tr>
<td>(Kim et al., 2015)</td>
<td><em>in vitro</em></td>
<td>Mouse fibroblast (L929 and HGF-1)</td>
<td>660; 2.5, 5.5 or 8.5</td>
</tr>
<tr>
<td>(Teuschl, Balmayor, Redl, van Griensven, &amp; Dungel, 2015)</td>
<td><em>in vitro</em></td>
<td>Myoblasts; fibroblasts; keratinocytes</td>
<td>470, 630; 30</td>
</tr>
<tr>
<td>(de Sousa et al., 2014)</td>
<td><em>in vivo</em></td>
<td>Mouse</td>
<td>846; 4</td>
</tr>
<tr>
<td>(Leite et al., 2014)</td>
<td><em>in vivo</em></td>
<td>Mouse</td>
<td>660-890; 3, 4</td>
</tr>
<tr>
<td>(Cheon, Kim, Lee, &amp; Kim, 2013)</td>
<td><em>in vivo</em></td>
<td>Mouse</td>
<td>470, 525 or 633; 3.55, 4.04 or 6.78</td>
</tr>
<tr>
<td>(Hsieh, 2013)</td>
<td><em>in vitro</em></td>
<td>Human foreskin fibroblasts</td>
<td>630; 3.4 or 6.8</td>
</tr>
<tr>
<td>(Paraguassú et al., 2014)</td>
<td><em>in vivo</em></td>
<td>Mouse</td>
<td>630; 24</td>
</tr>
</tbody>
</table>
(Fushimi et al., 2012) \textit{in vitro:} Human dermal fibroblasts; \textit{in vivo:} Mouse

456, 518 or 638 nm; 0.2, 0.3 or 0.6 J/cm$^2$; 0.3, 0.25 or 0.65 mW/cm$^2$; 1200 seconds; 1 day

\textit{In vivo}, green and red LEDs promoted greater cell migration. Cytokines increased expression with the use of the green LED increased expression of leptin, IL-8, HGF, KGF, TGF-\(\alpha\) and VEGF. The red LED increased HGF, KGF and TGF-\(\alpha\). The blue LED increased HGF and KGF. \textit{In vivo}, the red or green LEDs accelerate wound healing.

(Adamskaya et al., 2011) \textit{in vivo:} Mouse

470 or 629 nm; 50 mW/cm$^2$; 10 seconds; 1 day

Blue LED significantly accelerated healing when compared to the non-irradiated group. The red LED showed no healing effect.

(de Sousa et al., 2011) \textit{in vivo:} Mouse

700nm; 5, 10 or 15 6 J/cm$^2$; 8 mW/cm$^2$; 642, 1252 or 1878 seconds; 2, 4 or 6 days

The LED promoted lower expression of TGF-beta on day 2, with no difference in later days.

(Komine et al., 2010) \textit{in vitro:} Fibroblastos murino

627 nm; 4 J/cm$^2$; 25 mW/cm$^2$; 160 seconds; 1 day

LED induced proliferation and increased autocrine production of PDGF-C and activated the ERK signaling pathway through PDGF receptor phosphorylation.

(de Sousa et al., 2010) \textit{in vivo:} Mouse

460, 530 or 700 nm; 10 J/cm$^2$; 3.98, 7.46 or 10.94 mW/cm$^2$; 7, 11, 20 seconds; 7 days

The red and green LED showed a positive biomodulatory effect on fibroblasts when compared to the non-irradiated group.

(Dall Agnol, Nicolau, de Lima, & Munin, 2009) \textit{in vivo:} Mouse

640 nm; 6 J/cm$^2$; 30 mW/cm$^2$; 100 seconds; 1 day

LED promoted a slight acceleration of healing in diabetic and non-diabetic animals when compared to non-irradiated groups.
Corazza, Jorge, Kurachi, & Bagnato, (2007) in vivo: Mouse 635 nm; 5 or 10 J/cm$^2$; 90 mW/cm$^2$; 122 or 393 seconds; 21 days. The LED promoted the increase of blood vessels. The 5 J/cm$^2$ dose was better than the 20 J/cm$^2$ dose in the final stages of treatment.

Vinck, Cagnie, Cornelissen, Declercq, & Cambier, (2005) in vitro: Embryonic chicken fibroblasts 570, 660 or 950 nm; 0.1 J/cm$^2$; 10 mW/cm$^2$; 180 seconds; 3 days. The LED increased cell proliferation significantly compared to control.

Song et al., (2003) in vitro: Human fibroblasts 628 nm; 0, 0.44, 0.88, 2, 4.4 or 8.68 J/cm$^2$; 11.46 mW/cm$^2$; 3 days. Proliferation was more stimulated with a dose of 0.88 J/cm$^2$. Modulation in the expression of 111 genes related to wound healing was identified.

Vinck, Cagnie, Cornelissen, Declercq, & Cambier, (2003) in vitro: Fibroblasts of old chicken embryos 570, 660 or 950 nm; 0.1 or 0.53 J/cm$^2$; 10, 80 or 160 mW/cm$^2$; 1, 2 or 3 seconds; 3 days. All wavelengths stimulated cell proliferation.

Cha, Hur, Pak, Hong, & Suh, (2024) In vivo: Mouse 632 nm; 20 or 40 J/cm$^2$; 11.1 or 22.2 mW/cm$^2$; 1800 seconds; 14 days. The percentage wound closure, neopidermal length, collagen deposition and the number of microvessels were significantly higher in wounds treated with 22.2 mW/cm$^2$.

Table 2 - Risk of bias summary

| Study                              | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item | Syrcle Item |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| (Gomes et al., 2016)              | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Mo et al., 2019)                 | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (de Sousa et al., 2014)           | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Leite et al., 2014)              | Y           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Cheon et al., 2013)              | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Paraguassú et al., 2014)         | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Fushimi et al., 2012)            | Y           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Adamskaya et al., 2011)          | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (de Sousa et al., 2011)           | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (de Sousa et al., 2010)           | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Dall Agnol et al., 2009)         | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Corazza et al., 2007)            | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
| (Cha et al., 2024)                | U           | Y           | Y           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           | U           |
U, unclear; Y, yes; N, no. SYRCLE Items: 1, sequence generation; 2, baseline characteristics; 3, allocation concealment; 4, random housing; 5, blinded animal intervention; 6, random outcome assessment; 7, blinding outcome assessors; 8, incomplete outcome data; 9, selective outcome reporting; 10, other types of bias.