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Review

Healing Effects of Photobiomodulation on Diabetic Wounds

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Abstract: Diabetic patients frequently develop chronic ulcers of the lower extremities, which are a frequent cause for hospitalization and amputation, placing strain on patients, their families, and healthcare systems. Present therapies remain a challenge, with high recurrence rates. Photobiomodulation (PBM), which is the non-invasive application of light at specific wavelengths, has been shown to speed up healing of chronic wounds, including diabetic foot ulcers (DFUs). PBM produces photophysical and photochemical changes within cells without eliciting thermal damage. It has been shown to promote tissue regeneration and speed up wound repair by reducing inflammation and oxidative stress, accelerating cell migration and proliferation, and promoting extracellular matrix production and release of essential growth factors. The shortage of rigorous, well-designed clinical trials makes it challenging to assess the scientific impact of PBM on DFUs, and lack of understanding of the underlying mechanisms also hinders the conventional use of this therapy. This review gives a glimpse into diabetic wound healing and PBM, and the effects of PBM on diabetic wound healing.

Keywords: photobiomodulation; laser therapy; diabetes mellitus; diabetes; wound healing; chronic ulcers

1. Introduction

The number of diabetic patients worldwide is on the rise, with a 2017 estimated global prevalence of 8.8% aged between 20 and 79 years (424.9 million), which is expected to increase to 9.9% (628.6 million) by the year 2045 [1]. Approximately 4.0 (3.2–5.0) million people aged between 20 and 79 years are estimated to have died from diabetes mellitus (DM) in 2017; that is equivalent to one death every eight seconds [1]. Diabetes in all forms imposes an excessively high human, social, and economic cost on all income level countries. In 2017, the total healthcare expenditure by people with DM stood at US dollars(USD) 727 billion for those aged 20–79 [1].

Foot complications are among the most serious, debilitating, and costly complications of DM. Patients with DM commonly develop chronic, slow-to-heal ulcers that affect the lower extremities. These chronic wounds are a common and frequent cause for hospitalization and amputation, leading not only to incapacity and decreased quality of life, but also affecting psychological wellbeing, contributing to depression and placing financial strain on individuals, families, and healthcare systems. In 2007, one-third of diabetes costs were estimated to be linked to diabetic foot ulcers (DFUs), and currently patients experience health expenditure five times higher than those without foot ulcers [1]. DFUs are the most frequent cause of non-traumatic lower limb amputation [2], resulting in not only limb loss but also contributing to a 3-year mortality rate of 75.9% [3]. The International Diabetes Federation [1] estimates that a lower limb or part thereof is lost to amputation somewhere in the world as a consequence of diabetes every 30 s. It has been approximated that diabetic patients have a 25% lifetime risk of developing a foot ulcer, and are 100 times more likely to suffer from lower extremity amputation than

euglycemic patients [4]. Once an amputation has occurred, half of patients will develop an ulcer in the contralateral limb within 5 years [5], placing a further burden on patients. This personal and financial burden is expected to increase along with the anticipated increase in the prevalence of DM.

Current treatments for DFUs rely on patient education, prevention, early diagnosis, and comprehensive management [2]. Current therapies remain a challenge, with high recurrence rates. Photobiomodulation (PBM) has been shown to be beneficial in the treatment of a variety of medical conditions and pathologies, including chronic wounds and DFUs. PBM is defined as a mechanism by which nonionizing optical radiation in the visible and near-infrared (NIR) spectral range is absorbed by endogenous chromophores to elicit photophysical and photochemical events at various biological scales without eliciting thermal damage. Photobiomodulation therapy (PBMT) is defined as a photon therapy based on the principles of PBM [6]. Due to the need for the development of a more rapid, productive, cost-effective, and appropriate therapy to facilitate healing of chronic wounds, particularly DFUs, the use and further investigation of PBM is warranted.

2. Diabetic Wound Healing

Chronic wounds rarely occur in healthy individuals and frequently occur as a comorbidity with other diseases and conditions, such as DM, obesity, and spinal cord injury. A chronic wound is one which has failed to proceed through an orderly and timely reparative process. These wounds often become stuck in the inflammatory phase of healing and typically do not heal within three months. Diabetic ulcers of the lower extremities occur as a common complication of DM and involves a multifactorial pathogenesis including peripheral neuropathy and peripheral vascular disease, repetitive external trauma to the feet, and infection [7]. Most infected DFUs require some surgical intervention, ranging from minor to major interventions, including debridement and amputation, respectively. Infected DFUs are a major cause of lengthy hospital admission and contribute to more than a million amputations per year [8], with a 50% 5-year mortality rate amongst diabetic amputees [9].

Wound healing in diabetes is impaired by extrinsic and intrinsic factors. Extrinsic factors include repeated trauma or mechanical stress to the foot. Intrinsic factors play a major role in the development of DFUs. Hyperglycemia leads to the formation of advanced glycation end-products (AGEs) which prompt the production of inflammatory cytokines. There is also a decrease in collagen production and other essential extracellular matrix (ECM) proteins, and an increase in their destruction by matrix metalloproteinases (MMPs). There are also alterations in cellular morphology, abnormal differentiation of keratinocytes and fibroblasts, decreased cellular proliferation, altered immune function, and altered bioavailability of cytokines and growth factors [10]. There are also conditions of hypoxia, which impact negatively on wound healing.

Treatment of DFUs is centered on eliminating infection, the use of dressings to maintain a moist wound bed and to absorb exudate, offloading high pressure from the wound bed, and debridement to accelerate endogenous healing and facilitate the effectiveness of topically applied substances [11]. With the advancement of technology, new treatments for diabetic ulcers have been developed and include the incorporation of nanoparticles and growth factors into wound dressings, tissue-engineered human skin equivalents, stem cell therapy, vacuum-assisted closure (VAC) devices, hyperbaric oxygen therapy (HBOT), and gene therapy. Advances in the area of photonics and biophotonics has led to the efficacious use of light in the treatment of diabetic wounds.

3. Photobiomodulation (PBM)

The use of light as a therapy dates back to ancient civilizations, with ancient Egyptians and Indians making use of sunlight (heliotherapy) for healing and promoting health. Professor Endre Mester reported the earliest application of PBM in 1967. He described how irradiation accelerated hair growth on the back of shaven mice [12]. This piqued his interest, and he went on to show how PBM could stimulate wound healing in mice [13] and in human patients [14,15]. The use of light as a treatment has

subsequently evolved and is used to reduce inflammation and edema, to treat neurological disorders and pain, and to promote healing of various tissue types.

PBM involves the use of nonionizing forms of light from sources including lasers, light-emitting diodes (LEDs), and broadband light in the visible and near-infrared (NIR) spectra to cause physiological changes and therapeutic benefits. It is a non-invasive phototherapy that can be used alone or in combination with other wound treatments. PBM produces photophysical and photochemical changes within cells without eliciting thermal damage. Despite the fact that PBM is applied to treat a wide variety of ailments, it remains underutilized and controversial. This is mainly due to the poor understanding of the underlying cellular and molecular mechanisms, so its use is largely experimental. Another contributing factor is the fact that a large number of settings and parameters, such as wavelength (nanometers, nm), fluence (joules per square centimeters, J/cm^2), power density (watts per square centimeters, W/cm^2), pulse structure (nanoseconds, ns), and timing (seconds, s) of the applied light must be carefully chosen for each treatment. A less than optimal choice of these parameters results in unsatisfactory findings or even a negative therapeutic outcome [16]. There is almost a complete lack of reports of side effects or adverse events associated with PBM [16], something which counts largely in its favor.

Light that falls within the so called "optical window" at red and NIR wavelengths (600 to 1070 nm) are typically used for PBM [16]. The lower wavelengths in the red spectrum (600 to 700 nm) do not penetrate into tissue as deeply and are used to treat superficial tissue, while longer wavelengths (780 to 950 nm) penetrate much deeper and are used to treat deeper-seated tissues. The application of light in the blue/violet spectrum has been shown to suppress pathogenic bacterial growth. The power of light used typically lies in the range of 1 to 1000 mW and depends on the application. The dose (or fluence), which is a function of the combination of irradiance (medicine) and time (dose), is also very important, and also varies depending on the application [16].

3.1. Mechanisms of PBM

The precise mechanisms of action of PBM are not fully known and understood, however a broad range of effects at the molecular, cellular, and tissular levels have been observed. What is known is that PBM appears to have a profound effect on the cellular mitochondria where the photons are absorbed by various components of the respiratory chain, primarily complex IV or cytochrome c oxidase (Cox), which transfers electrons from cytochrome c to molecular oxygen [17–19]. The photoreactivity of Cox is due to its four metal centers; two heme moieties (heme a and heme a3) and two redox-active copper sites (CuA and CuB) [20].

Karu and colleagues [21] revealed that the redox state of Cox is influenced by red light, and is dependent on the initial redox state at the time of irradiation. It was also shown that irradiation intensified the transfer of electrons in Cox, resulting in accelerated oxidative phosphorylation [20]. An increase in other electron transfer complexes has also been observed. Yu and co-workers [22] found an increase in enzyme activity of complex I, III, and IV in irradiated isolated mitochondria (660 nm; 10 mW/cm^2; 0.6, 1.2, 2.4, and 4.8 J/cm^2). Masha et al. [23] found up-regulation of genes coding for complex I, IV, and V following irradiation at 660 nm (11 mW/cm^2, 5 J/cm^2). The effect on mitochondria results in increased adenosine triphosphate (ATP) synthesis [19,24], proton electrochemical potential [24,25], and oxygen consumption, as well Nicotinamide adenine dinucleotide (NADH) synthesis [26]. The increase in ATP has been found to peak immediately in irradiated mouse embryonic fibroblasts (810 nm; 0.3, 3, and 30 J/cm^2) and decline to baseline levels over 6 h [27]. Increased levels of ATP following PBM have also been found under hypoxic conditions [28–30], which normally results in decreased ATP synthesis [29].

There is also modulation of intracellular reactive oxygen species (ROS), which are involved in cell signaling pathways and gene transcription. Under normal physiological conditions, ROS are produced during the synthesis of ATP, and since PBM boosts oxygen metabolism, it also acts to increase ROS production [16]. PBM also displaces nitric oxide (NO) from Cox. NO inhibits mitochondrial respiration

by binding to the heme iron:copper binuclear center (a3/CuB) of Cox, thus displacing oxygen [31,32]. This photodissociation allows for the influx of oxygen and resumption of respiration [31,33]. It has also been hypothesized that PBM may also dissociate NO from intracellular stores, such as nitrosylated hemoglobin and myoglobin, leading to vasodilation [34]. Laser irradiation (830 nm, 4.4 mW/cm^2, 5 J/cm^2) of human skin fibroblast cells resulted in an increase in ROS and NO 15 min post-irradiation in what would appear to be a direct photochemical effect [35]. Chen et al. [27] demonstrated an increase in mitochondrial ROS fluorescence in irradiated (810 nm; 1–30 mW/cm^2; 0.3, 3, and 30 J/cm^2) murine embryonic fibroblasts. Pal et al. [36] found that the generation of ROS in irradiated (632.8 nm, 0.5 to 16 J/cm^2, 0.64 to 1.16 mW/cm^2 for whole cell culture irradiation; and 330 mW/cm^2 to 20 W/cm^2 for single cell irradiation) human skin fibroblasts was dependent on laser fluence and not on laser intensity. Zhang et al. [30] showed that PBM increased intracellular NO in irradiated cardiac cells (670 nm, 25 mW/cm^2, 7.5 J/cm^2), an effect which was no longer evident when NO scavengers were added and partially impeded by nitric oxide synthase (NOS) inhibitors. Zhang et al. [30] came to the conclusion that the increase in NO was due to NOS and a second unidentified source, possibly photodissociation from Cox.

When it comes to irradiation and destruction of bacteria with blue/violet light, the mechanism of action centers around the production of high amounts of ROS. Irradiated light is absorbed by photoacceptors within the bacterium such as porphyrins and flavins [37]. These phorphyrins absorb the photon energy, become excited, and jump to the triplet state. They then release the extra energy and pass it onto molecular oxygen creating ROS, which interact with numerous macromolecules within the cell causing cellular damage and leading to cell death [38].

3.2. Cellular Effects of PBM

PBM has been shown to affect cell functions, such as viability, proliferation, migration, and metabolism, in a variety of cell types, including fibroblasts, mast cells, osteoblasts, Schwann cells, stem cells, keratinocytes, and smooth muscle cells. It has been shown to promote tissue regeneration and speed up wound repair by reducing inflammation and stimulating cell migration and proliferation, ECM production, and release of essential growth factors, and increasing the mean breaking strength of the wound.

Amaroli and colleagues [39] evaluated various cellular responses in human endothelial cells (HECV) irradiated with an 808 nm diode laser (1 W/cm^2, 60 J/cm^2; or 0.95 W/cm^2, 57 J/cm^2). Cellular viability, free radical-induced oxidative stress, nuclear factor kappa B (NF-κB) activation, NO release, mitochondrial respiration, and wound healing repair were measured. Irradiated cells demonstrated increased proliferation and migration coupled with a moderate increase in ROS production without a significant increase in oxidative stress and oxidative stress-activated processes. PBM stimulated mitochondrial oxygen consumption and ATP production. There was no effect on cellular viability, however, PBM led to an increased wound healing rate. Their results demonstrated that NIR light led to a shift from anaerobic to aerobic metabolism. Assis et al. [40] also showed PBM was effective in modulating oxidative stress and reducing inflammation in injured muscle. Irradiation (808 nm, 3.8 mW/cm^2, total energy 1.4 J) not only lessened oxidative and nitrative stress, but also reduced lipid peroxidation, nitrotyrosine formation, NO production, and the inflammatory response (NF-κB, COX-2, TNF-α and interleukin(IL)-1β) and amplified superoxide dismutase (SOD) gene expression. Otterço and colleagues [41] irradiated wounded rats with a wavelength of 670 nm (30 mW, 14.28 J/cm^2) for 15 consecutive days. Histopathological analysis revealed a lower inflammatory infiltrate, as well as increased collagen. There was an increase in vascular endothelial growth factor (VEGF) and a decrease in tumor necrosis factor-alpha (TNF-α).

Growth factors have an important role during wound repair and are involved in regulating cell growth, division, differentiation, and migration, and are also concerned with various signaling pathways. Numerous studies have shown the beneficial effect of PBM on the increased production of various growth factors essential to wound healing. Damante and colleagues [42] showed that

irradiation of human gingival fibroblasts by an infrared laser (780 nm; 1 W/cm^2; 3 and 5 J/cm^2) led to the increased production and secretion of basic fibroblast growth factor (bFGF). Jere et al. [43] demonstrated an increase in cell migration rate, proliferation, and viability, as well as an increased release of epidermal growth factor (EGF) in wounded fibroblast cells, which lead to activation of the JAK/STAT signaling pathway.

The effect of PBM in aged animals has also been shown to be effective. Fiorio et al. [44] investigated PBMT (660 nm, 1.07 W/cm^2, 72 J/cm^2) in cutaneous wound healing in aged rats (500 days). The study demonstrated that PBM is effective in the modulation of inflammatory mediators (IL-6, CINC-1, and VEGF) and matrix metalloproteinases and their inhibitors (MMP-3, MMP-9, and TIMP-2). There was also increased collagen production during different phases of tissue regeneration.

3.3. Effects of PBM Using Blue Light on Bacterial Growth and Fibroblasts

PBM has been shown to eradicate bacteria within the blue spectrum of light. Lipovsky and colleagues [37] irradiated *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) with a halogen lamp with filters for irradiation in the blue (400–500 nm) and red (500–800 nm) spectra, or with blue LEDs (415 and 455 nm; 100 mW/cm^2; for 30, 60, and 120 J/cm^2). There was more ROS production in bacterial cells exposed to blue light (400–500 nm) than those exposed to red light (500–800 nm). When irradiated with LEDs, 415 nm was found to be more valuable than a wavelength of 455 nm, at higher fluences. A fluence of 30 J/cm^2 at 415 nm and a fluence of either 30 or 60 J/cm^2 at a wavelength of 455 nm resulted in an increase in proliferation of *S. aureus*.

Enwemeka and colleagues [45] showed that exposure of two strains of methicillin-resistant *Staphylococcus aureus* (MRSA) to blue light at a wavelength of 470 nm (output power of 150 mW; irradiance of 30 mW/cm^2) was able to inhibit and kill the bacteria. Two strains of MRSA were used, IS-853 representing hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and US-300 representing community-acquired *S. aureus* (CA-MRSA). Quantification of bacterial colonies and aggregate area of colonies 24 h post-irradiation showed that there was a dose-dependent reduction in both strains. A low fluence of 3 J/cm^2 produced 34.1% and 27.6% death for US-300 and IS-853 strains, respectively, which increased to more than 48% and 67.3% at 7 J/cm^2, respectively. When a fluence of 11 J/cm^2 was provided, a reduction of 61.2% and 56.4% was observed, respectively. Over 80% bacterial death was noted in both strains when a fluence of 35 J/cm^2 was used, and there was an average death of 90.4% for both strains at a fluence of 55 J/cm^2, and 91.7% and 94.8% of the aggregate area was eradiated for US-300 and IS-853 strains, respectively. In a similar study, using the same LED light device (470 nm wavelength; output power of 150 mW; irradiance of 30 mW/cm^2) Bumah and colleagues [46] irradiated MRSA USA300 three times (with 30 min between exposures) to a fluence of 36 J/cm^2 (cumulative fluence of 108 J/cm^2) or four times (with 30 min between exposures) to a fluence of 65.5 J/cm^2 (cumulative fluence of 262 J/cm^2). MRSA treated with blue light at either exposure regimens did not express any bacterial growth. FTIR analysis showed that there were changes in DNA conformation and that irradiation of MRSA with 470 nm light induces A-DNA cleavage. Biener and colleagues [38] also showed that irradiation of MRSA USA300 to a diode laser with a wavelength of 405 nm (output power of 500 mW; irradiance of 135 mW/cm^2) inhibited bacterial growth. Either bacterial cells were irradiated with a single dose, or a double dose with 30 min between doses; each dose was at a fluence of 121 J/cm^2. A double dose was found to be more efficient than a single irradiation. They also showed that there was depolarization of the cell membrane and that MRSA expressed demonstrable amounts of porphyrins, and its production was dependent on the cell cycle phase. It was suggested that blue light was able to target these porphyrins, and due to the production of ROS and attack thereof of the membrane, there was a decrease in the transmembrane potential, leading to cell death.

An important paper published by Masson-Meyers et al. [47] determined the effect of blue light on fibroblast cells. If blue light is to be used to treat infected wounds in vivo, the treatment protocol needs to have an inhibitory effect on the invading pathogen, while leaving the surrounding host cells intact. Cells were irradiated at a wavelength of 470 nm and different fluencies of 3, 55, 110,

or 220 J/cm^2 (output power of 150 mW; irradiance of 30 mW/cm^2). Four different assays were carried out to determine cellular viability 24 h post-irradiation. The MTT assay showed that irradiation with 55, 110 and 220 J/cm^2 significantly impairs mitochondrial activities and decreases fibroblast viability. When cellular viability was determined by the neutral red assay as well as the Trypan blue assay, a significant decrease was observed following irradiation with 110 or 220 J/cm^2, indicating a disruption to the cell membrane integrity. Data attained from the live/dead fluorescence assay showed only slight, insignificant decreases in cell viability at all fluences tested. Overall, these results showed that there was a dose response in fibroblast cells in response to irradiation at 470 nm, with fluences above 110 J/cm^2 becoming intolerable to cells. Opländer and colleagues [48] found that irradiation of fibroblasts at wavelengths of 410, 420, 453, and 480 nm (irradiance of 50 mW/cm^2) at different fluences produced different results. Fibroblast numbers were significantly decreased when irradiated with 410 nm and 420 nm at 60 and 90 J/cm^2, whereas irradiation with 480 nm resulted in a significant increase at 30 and 60 J/cm^2. Irradiation with 453 nm produced no difference. Irradiations with 410 nm and 420 nm at a fluence of 10 J/cm^2 resulted in increased intracellular oxidative stress, while wavelengths of 435 nm and 480 nm produced no effect at the same fluence. This increase in oxidative stress was partly due to an increase in the production of singlet oxygen. Irradiations at 410 nm, 420 nm, and 435 nm significantly reduced cellular proliferation when cells were irradiated daily with 10 J/cm^2 over 4 days, while irradiations with 480 nm had no effect on cellular proliferation.

Since PBM in the blue spectrum has been found to be lethal to bacterial growth, there is promise for this therapy to be used in the treatment of infected wounds, however, more detailed in vivo studies on infected wounds need to be conducted. An advantage of such a treatment is that there are no external drugs involved, and it is highly unlikely that bacteria will develop resistance to this kind of treatment. Caution should still prevail, however, as some wavelengths and fluences have been shown to increase bacterial cell proliferation, and the effects on fibroblasts are not well established and vary according to wavelengths used. Despite this, PBM using blue light may be a viable alternative to drug treatment.

4. Photobiomodulation for Diabetic Wound Healing

Various studies have found PBM to be beneficial to diabetic wound healing (Table 1). PBM has promoted and sped up repair in non-healing ulcers. Al-Watban [49] reported on the use of different lasers with wavelengths in the visible to NIR spectra (532, 633, 810, 980, and 10,600 nm) and polychromatic LED clusters (510–872 nm, visible to infrared). Streptozotocin-induced Sprague-Dawley rats were subjected to a full-thickness wound (102.5 ± 9 mm^2) or a burn (148 ± 12.5 mm^2) and then treated with PBM three times a week at different fluencies (5, 10, 20, and 30 J/cm^2). It was concluded that the best treatment option for both diabetic wounds and burns was with a laser at a wavelength of 633 nm with 38.5% and 53.4% improvements, respectively. A dose (fluence) of 4.71 J/cm^2 for diabetic burns, and 2.35 J/cm^2 per dose for diabetic wound healing administered three times a week was recommended. Eissa and Salih [50] irradiated diabetic wounded rats (632.8 nm, 4 mW/cm^2) five times a week until the wounds healed. Irradiated diabetic wounds took 21 days to heal, whereas non-treated control wounds took 40 to 60 days to heal.

Chronic diabetic wounds have shown decreased levels of cytokines and growth factors essential to wound healing [51]. PBM has been show to promote the synthesis and release of some of these under diabetic conditions, including EGF, IL-6, bFGF, platelet-derived growth factor (PDGF), and transforming growth factor beta 1 (TGF-β1). Jere et al. [43] demonstrated an increase in cell migration rate, proliferation, and viability in diabetic induced wounded fibroblast cells, which was ascribed to increased EGF, which in turn lead to activation of the receptor (EGFR) and the JAK/STAT pathway. They concluded that PBM at 660 nm (11 mW/cm^2, 5 J/cm^2) is able to intensify and regulate cellular autocrine signaling, leading to increased cell proliferation and migration. Esmaeelinejad and Bayat [52] irradiated human skin fibroblasts (632.8 nm; 0.66 mW/cm^2; 0.5, 1, and 2 J/cm^2) in media that had different glucose concentrations (5.5 (physiological levels), 11.1, and 15 mM/L) and observed an

increase in the release of intracellular cytokines IL-6 (at 0.5, 1, and 2 J/cm^2) and bFGF (at 2 J/cm^2). Khoo et al. [53] showed that PBM significantly augmented PDGF and up-regulated gene expression of FGF in diabetic mice skin fibroblasts when irradiated at a wavelength of 810 nm (10 mW/cm^2) with a fluence of 1 J/cm^2. Ma et al. [54] irradiated streptozotocin-induced Wistar rats to a wavelength of 630 nm (5, 10, and 20 mW/cm^2; 3.6 J/cm^2) and found that PBM significantly altered TGF-β1 and bFGF expression after 4 days. There was also an attenuation of the inflammatory response, greater reepithelization, mature granulation tissue (fibroblasts), and extensive collagen deposition, especially with irradiation of 20 mW/cm^2 [54].

Prolonged inflammation contributes to the pathophysiology of diabetic wound healing [55]. PBM has been shown to have anti-inflammatory effects under such conditions. Akyol and Güngörmüş [56] treated incisions made with a diode laser or a scalpel on the left side of the dorsum in streptozotocin-induced Wistar rats with PBM (808 nm, 0.1W/cm^2, 10 J/cm^2) for five sessions on alternative days. Rats were sacrificed at 10 (2 days after the last PBM treatment) and 20 days and the degree of reepithelialization and inflammation was investigated. There was a lower degree of reepithelialization and acute inflammation in the control group, with a significant increase in reepithelialization and diffuse acute inflammation in PBM treated groups. There was no statistically significant difference between the groups in inflammation and reepithelialization at day 20. PBM at 830 nm (5 J/cm^2, 4.4 mW/cm^2) resulted in a decrease in pro-inflammatory cytokines (TNF-α and IL-1β) and apoptosis in diabetic wounded fibroblast cells [35]. Irradiation of the same cells to 660 nm (11 mW/cm^2, 5 J/cm^2) resulted in decreased apoptosis and IL-1β [57]. There was also an increase in cellular viability and proliferation. Hypoxic cells also responded to PBM at the same parameters, and showed increased viability and proliferation, as well as decreased TNF-α [57].

During wound healing, the ECM is maintained by a balance between collagen production and ECM degradation to facilitate cellular migration and removal of debris. In the case of diabetes, this balance is negated; there is decreased ECM synthesis and increased ECM degradation [58]. Carvalho and colleagues [59] irradiated diabetic Wistar rats (660 nm, 4 J/cm^2) and found that PBM promoted healing by increasing collagen synthesis. Tatmatsu-Rocha et al. [60] irradiated 4 cm^2 wounds in diabetic mice daily for 5 days (superpulsed AsGa laser, 904 nm, 40 mW, 304.8 mW/cm^2, 18.288 J/cm^2). It was shown that there was increased collagen production, organization and angiogenesis, and decreased oxidative and nitrosative stress. Peplow et al. [61] irradiated wounded mice that represented a model of type 2 DM. Mice received a full thickness wound using a 5 mm punch. It was determined that PBM at a wavelength of 660 nm (4.7 to 6.3 J/cm^2, with 25, 50, or 100 mW) increased granulation tissue formation in diabetic mice when irradiated daily for 7 days. Lau et al. [62] ascertained irradiation of diabetic rats at 808 nm (5 J/cm^2) led to swifter wound contraction, and increased fibroblasts, granulation tissue, and collagen deposition. This occurred using three different power densities of 0.1, 0.2, and 0.3 W/cm^2, however, better results were seen at 0.1 W/cm^2. Aparecida Da Silva et al. [63] irradiated wounded diabetic rats to a wavelength of 660 nm (50 mW, 4 J/cm^2) and found that PBM reduced the genetic expression of proteinases MMP-2 and -9, and increased total collagen production, particularly collagen type-III. Ayuk et al. [64] showed increased collagen production when diabetic wounded fibroblast cells were irradiated at 660 nm (10.22 mW/cm^2, 5 J/cm^2). In similar studies, gene profiles showed that genes related to collagen (Collagen, type XI, and Collagen, type XIV) were up-regulated and various MMPs down-regulated (MMP-1, -2, -8, -12, -14, and -16) in response to laser irradiation. MMP inhibitors were also up-regulated (TIMP-1) [65]. When the same cells were irradiated at a different wavelength of 830 nm (10.76 mW/cm^2, 5 J/cm^2) PBM produced a stimulatory effect on various cell adhesion molecules, namely, cadherins, integrins, selectins and immunoglobulins [66].

A study on the effect of PBM on *S. aureus* infected wounds in diabetic rats was conducted by Ranjbar and colleagues [67]. Streptozotocin-induced male Wistar rats received 4 cm full-thickness linear incisions on the dorsal midline which were contaminated with 5×10^7 CFU/mL of *S. aureus*. The wounds were then closed with sutures. On the third day post-wounding, wounds were irradiated daily for 5 consecutive days using a laser with a wavelength of 685 nm (15 mW, 3 J/cm^2, spot of

0.028 cm^2); control rats were sham irradiated. PBM resulted in decreased bacterial numbers, with a significant decrease observed in the irradiated rats ($0.51 \times 10 \pm 0.2 \times 10$ CFU/mL) as compared to the controls ($8.4 \times 10^7 \pm 1.8 \times 10^7$ CFU/mL). PBM also resulted in significantly shorter wound length (on days 14 and 21), and a significant increase in the number of macrophages, new blood vessels, fibroblasts, and collagen deposition. The breaking strength of the scars was also significantly increased in PBM treated rats [67]. This study has shown the potential of utilizing PBM in the treatment of infected diabetic wounds.

Covering of wounds with a transparent dressing may be required when performing PBM on wounds, especially when performing PBMT by a contact procedure. This is done to minimize wound damage caused by the tip of the device probe, to prevent cross contamination if irradiating multiple wounds, and to maximize irradiation [68]. Chung and colleagues [68] tested methods for dressing full-thickness excisional wounds that would be suitable for use with PBM in diabetic and nondiabetic mice. The combined use of Tegaderm HP dressing and Cavilon (protects the skin from adhesive stripping) and Mastisol (exceptional adhesive properties to benzoin compound tincture) adhesive agents was shown to be efficient for covering wounds of diabetic mice for over 14 days. Irradiated wounds had a very high rate (>80%) of wound splinting (healing occurs mainly by reepithelization and granulation tissue formation) in diabetic mice, without recourse to invasive treatments, such as sutures. Tegaderm dressing has been shown to transmit 93% of laser light at a wavelength of 532 nm, 94% at 1064 nm, and 74% at 720–800 nm [69,70].

PBM has also been shown to be successful in human studies, with no reported side effects. Nteleki et al. [71] showed that PBM may be beneficial in the treatment of DFUs in combination with standard podiatric care and treatment. Patients with type 2 diabetes with a stable or worsening lower extremity ulcer that had been present for a minimum of 4 weeks were enrolled into a pilot study. Patients all received standard podiatric care, which consisted of wound cleaning and debridement, wound dressing, offloading, and infection control (antibiotics) if necessary. Patients were divided into three groups; group 1 received placebo PBM; group 2 received PBM of the ulcer; and group 3 received PBM of the ulcer and regional lymph nodes so as to increase lymph drainage. DFUs were irradiated twice a week for a maximum of 90 days with a LED cluster probe (630 and 850 nm). Ulcers were irradiated after podiatric treatment and before dressing. Wounds in group 2 and 3 healed at a far more rapid rate than wounds in group 1. The results of the study showed that PBM has the potential to stimulate and increase healing rates in combination with podiatric medicine.

Mathur and colleagues [72] irradiated (660 nm, 3 J/cm^2, 50 mW/cm^2) non-infected grade I diabetic foot ulcers together with conventional treatments. Wound reduction was measured at two weeks and was found to be significantly reduced in 75% of PBM treated ulcers (30–50%); control wounds showed a decrease in wound area of less than 20% in approximately 80% of ulcers. There was also increased granulation tissue compared to control patients. Kaviani et al. [73] performed a randomized double-blind controlled clinical trial and treated stage I and II DFUs (685 nm, 50 mW/cm^2, 10 J/cm^2) six times per week for two weeks, and then every other day until healed. It was found that there was a reduction in ulcer size by the 2nd and 4th week. A similar study showed a decrease in ulcer size in type 2 diabetic patients (40.24% as compared with 11.87% for the control group) [74]. Ruh et al. [75] treated pressure ulcers, classified as degree III and IV according to the National Pressure Ulcer Advisory Panel (NPUAP), with PBM at 660 nm (100 mW, 2 J/cm^2) once a day, with intervals of 24 h, for a total of 12 applications. Wound closure analysis revealed improvement of the granulation tissue size up to 50%, and gene analysis of ulcer border tissue obtained through biopsy showed a down-regulation in TN-Fα and up-regulation in VEFG and TGF-β. It should be noted that this study did not include controls, however, despite this, PBM still showed promise of being beneficial to diabetic wound healing.

Table 1. Effect of Photobiomodulation (PBM) on diabetic wound healing.

Wavelength (nm)		Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
Lasers	532	5, 10, 20, and 30	20.4 (532 nm)	-	Three times a week.	STZ-induced Sprague-Dawley rats. Full-thickness wounds 102.5 ± 9 mm^2, or a burn (148 ± 12.5 mm^2).	Wound- or burn-healing percentages.	Significant wound (38.5%) and burn healing (53.4%) were demonstrated at 633 nm with 4.71 J/cm^2 (actual dose)* for wounds and 2.35 J/cm^2 (actual dose) for burns.	[49]
	633		15.56 (633 nm)						
	810		22.22 (810 nm)						
	980		22.22 (980 nm)						
	10,600		66.37 (10,600 nm)						
LED cluster	510–872		13.6 (LED cluster)						
632.8		-	4	-	Five times a week until healed.	Alloxan-induced Wistar rats with full-thickness, excisional wound (diameter 2.5 ± 0.2 cm) on the dorsum.	Wound diameters.	Wounds healed within 21 days in irradiated animals, and took up to 60 days to heal in the controls.	[50]
660		5	11	-	Irradiated once and left to incubate for 24 h.	Normal, wounded (scratch assay), diabetic (hyperglycemia, 22.6 mMol/L) and diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L) fibroblast cell models.	Cellular migration, proliferation, viability, EGF, p-EGFR, p-JAK2, p-STAT1 and p-STAT5.	Increased migration rate in wounded and diabetic wounded models. Increased proliferation and viability in all models. Increased EGF and p-JAK2 in wounded, diabetic and diabetic wounded models, and increased p-EGFR, p-STAT1 and p-STAT5 in all models.	[43]
632.8		0.5, 1, and 2	0.66	-	Cells irradiated once daily for three consecutive days.	Human skin fibroblasts grown under hyperglycemic (11.1, and 15 mM/L glucose) conditions.	IL-6 and bFGF	0.5, 1, and 2 J/cm^2 stimulates the release of IL-6 from fibroblasts cultured in hyperglycemic (15 mM/L) media. 2 J/cm^2 significantly increased the release of bFGF from fibroblasts cultured in hyperglycemic (11.1 ad 15 mM/L) media.	[52]
810		1	10	-	Single irradiation and incubated for 1 h.	Skin fibroblasts isolated from male Balb/c mice.	RT-qPCR for VEGF, EGF, PDGF, FGF.	Significant increase in FGF expression; PDGF expression increased, but was not significant.	[53]

Table 1. *Cont.*

Wavelength (nm)	Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
630	3.6	5, 10, and 20	-	Five times a week for two weeks.	STZ-induced male Wistar rats with two 100 cm^2 incisions each side on the dorsum.	Percentage wound closure, histology (PMNL, reepithelization, fibroblasts, new vessels, and collagen synthesis), bFGF and TGF-β1.	Wounds irradiated with 20 mW/cm^2 closed significantly faster at 3 days as compared to controls, while at 6 and 9 days all three PBM groups closed significantly faster, while at 12 days there were no differences. Histology showed increased collagen fibers at day 4 in wounds irradiated with 10 and 20 mW/cm^2. At day 8, there was attenuated inflammation, mature granulation tissue, extensive collagen deposition, and greater reepithelization in all the PBM groups, while the control group showed more inflammatory exudate and fresh granulation tissue. At day 14, almost all the wounds in all PBM and control groups were covered by new epithelial cells, granulation tissue was replaced with fibrous scar, fibroblasts decreased, and intercellular collagen content increased. There was an increase in new vessels in wounds irradiated with 5 and 20 mW/cm^2. bFGF significantly increased in all three PBM groups, but was not evident at 14 days. Wounds irradiated with 10 mW/cm^2 showed significant increase in TGF-β1 at 4 days.	[54]
808	2 (Total of 10)	100 (PBM)	-	Irradiated for five sessions	STZ-induced Wistar rats received one 15 cm long incision on the left hand side (group 3) and one on the right hand side (group 2), both induced by a laser, and one incision on the right hand side induced by a scalpel (group 1). Wounds were sutured and single wound on left hand side received PBM (group 3).	Histology slides (hematoxylin-eosin) examined and reepithelialization and inflammation graded 1 to 4.	At day 10 there was increased reepithelization in groups 2 and 3, and acute inflammation in groups 1 and 2. In the group 3 (PBM treated), fibroblast proliferation was evident. There were no differences at day 20.	[56]

Table 1. *Cont.*

Wavelength (nm)	Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
830	5	4.4	-	Irradiated once and left to incubate for various times (15 min; 1 h; 24 h; 48 h)	Normal, wounded (scratch assay), diabetic (hyperglycemia, 22.6 mMol/L) and diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L) fibroblast cell models.	Proliferation, apoptosis, viability, NO, ROS, cytokines (TNF-α; IL-1β; IL-6)	PBM had no effect on cellular viability (above 95%), and significantly increased proliferation at 24 h and 48 h in normal wounded (51% and 19%, respectively) and diabetic cells (53% and 28% respectively). Twenty-four hours post-irradiation there was a significant decrease in apoptosis in normal wounded (82%) and diabetic wounded cells (31%). Significant decrease in all cell models in TNF-α 1 h post-irradiation was observed. This decrease was still evident in normal wounded and diabetic wounded cells at 24 h. IL-1β was decreased in normal cells at 1 h and in diabetic wounded cells at 24 h. There were no significant changes in IL-6. There was a rapid significant increase in NO in all irradiated cells, which was seen 15 in post-irradiation but not at 1 h. There was also more ROS in irradiated cells 15 min post-irradiation.	[35]
636	5	11	-	Irradiated once and left for 1 h or 24 h.	Normal, wounded (scratch assay), diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L), and hypoxic (without FBS for 24 h and anaerobic incubation for 24 h.	Morphology, viability, proliferation, apoptosis, pro-inflammatory cytokines (TNF-a, IL-1β, and IL-6) and NF-kB translocation.	A high rate of migration into the central scratch was observed post-PBM, and hypoxic cells regained their typical morphology. Normal wounded cells exhibited increased proliferation (1 h and 24 h), and decreased apoptosis (1 h and 24 h), TNF-α (1 h) and IL-1β (1 h and 24 h). There was a significant increase in viability (24 h) and proliferation (1 h and 24 h), and a decrease in apoptosis (1 h) and IL-1β (1 h and 24 h) in irradiated diabetic wounded cells. There was a significant increase in cellular viability and proliferation (1 h and 24 h), and decreased apoptosis (24 h) and TNF-α (24 h), and an increase in IL-6 in irradiated hypoxic cell models. All cell models exhibited NF-kB nuclear translocation 1 h post-PBM.	[57]
660	10	–	-	Treated daily for 3, 7 or 14 days.	Alloxan-induced adult male albino Wistar rats with 8 mm diameter punch wound.	Histology (hematoxylin-eosin and Masson's trichrome staining, and immunohistochemical stains for macrophages).	There was significantly more collagen and less macrophages in PBM-treated wounds on days 3, 7 and 14 as compared to unirradiated control rats.	[59]

Table 1. *Cont.*

Wavelength (nm)	Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
904	18.288	304.8	9500 Hz with pulse duration of 60 s and 20% duty cycle	Daily for 5 consecutive days.	STZ-induced male Swiss mice with 4 cm^2 surgical wound in the posterior iliac crest reaching down to the hypodermis.	Histology (hematoxylin-eosin and Masson's trichrome staining). Biochemical determinations included TBARS (lipid peroxidation) catalase activity and nitrite concentration.	Histology results showed a fair number of fusiform fibroblasts and increased blood vessels in irradiated diabetic mice, with an intense deposition of a more organized collagen matrix. There was a significant reduction of nitrite levels and TBARS, and a significant increase in catalase activity.	[60]
660	4.7–6.3 (2 J/day)	58–78 (25 mW), 116–156 (50 mW), 233–313 (100 mW)	-	Daily for 7 consecutive days.	Diabetic mice (BKS.Cg-m þ/þ Leprdb/J), which were leptin receptor deficient and represented a model of type-2 diabetes, received full thickness circular wounds using a 5 mm diameter skin punch.	Histology slides (hematoxylin-eosin) graded (1–15), Photographic images were analyzed for dermal gap and epithelial gap.	All irradiations increased the extent of epithelial regrowth, granulation tissue, and collagen, and induced a greater inflammatory response.	[61]
808	5	100 (G1), 200 (G2), 300 (G3)	-	Daily for 9 consecutive days.	STZ-induced Sprague Dawley rats received full thickness circular wounds on the dorsal using a 6 mm punch.	Percentage wound contraction, and histology slides (hematoxylin-eosin).	Percentage wound contraction increased for all irradiated groups on days 3 and 6. Histology showed increased proliferation of epithelium near the wound surface, with denser connective tissue, angiogenesis, and intense inflammatory cells at day 3. By day 6, there was intense granulation tissue formation (G1) with a minimal to mild inflammatory infiltrate with restoration of epidermis on the wound surface. On day 9 the epidermis was covered in both irradiated mice and controls, with formation of stratified keratin at the superficial layer.	[62]
660	4	1430	-	-	STZ-induced male Wistar rats received full thickness circular wounds on the dorsal using an 8 mm punch.	Histology (hematoxylin-eosin and Masson's trichrome and Picrosirius Red staining) and RT-qPCR (MMP-2 and MMP-9)	Results showed increased, denser total collagen, with significant increases in type I. Genetic testing showed decreased expression of the proteinases MMP-2 and -9.	[63]
660	5	10.22	-	Irradiated once and left for 48 or 72 h.	Normal, and diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L).	Viability, proliferation, collagen type I.	Cellular viability was increased in irradiated diabetic wounded cells 48 h and 72 h post-PBM. Proliferation results showed an increase at 48 h and a decrease at 72 h (attributed to over confluence). There was a significant increase in collagen type I.	[64]
660	5	10.22	-	Irradiated once and left for 48.	Normal, wounded (scratch assay), diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L).	Gene Expression Profiling of 84 genes (RT-qPCR using gene array–extracellular matrix).	Genes related to Collagen, type XI and XIV were up-regulated, while MMP1, -2, -8, -12, -14, and -16 were down-regulated. TIMP1 was also up-regulated.	[65]

Table 1. *Cont.*

Wavelength (nm)	Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
830	5	10.76	-	Irradiated once and left for 48.	Normal, and diabetic wounded (scratch assay and hyperglycemia, 22.6 mMol/L).	Gene Expression Profiling of cellular adhesion molecules (RT-qPCR using gene array).	Up-regulation of selectin E, selectin L, vascular cell adhesion molecule 1, Sarcoglycan (epsilon), and versican.	[66]
685	3	535.7	-	Daily for five consecutive days.	STZ-induced male Wistar rats received a 4 cm full thickness linear incision on the lumbar region. Wounds were contaminated with 5 $\times 10^7$ CFU/mL of *S. aureus*, sutured and left for 48 h.	Macroscopic evaluation, bacteriological analysis, and histology.	Bacterial inhibition occurred following PBM, with significantly decreased bacterial numbers in PBM group (0.51×10^1 CFU/mL) compared to controls (8.4×10^7 CFU/mL). There was a significant decrease in wound length on days 14 and 21. PMB treated infected wounds had increased epithelialization, fibroblasts, collagen, neoangiogenesis and scar breaking strength.	[67]
LED cluster probe: 3x 630 nm LEDs and 8x 830 LEDs	3 per spot	75	Pulsed: continuous ratio 30:70%. Wound protocol: 292, 930, 1174, 1520, 1574, 1604, 4788, 6352 Hz. Muscle protocol (lymph nodes): 292, 588, 1520, 1604, 1756, 1760, 9396 Hz.	Irradiated treated twice a week (72 h between treatments) for a maximum of 90 days.	Adult patients with type 2 DM and lower limb ulcers of at least 4 weeks' duration. Patients were divided into three groups: placebo PBM (group 1–control); PBM of ulcer (group 2; PBM of ulcer and regional lymph nodes (group 3). All groups received standard podiatric care (cleaning, debridement, dressing, off-loading, and antibiotics if necessary).	Visual examination of wounds and digital photography (ulcer size and area granulation).	Combination of podiatric care and PBM yields the potential to stimulate and increase healing rates of chronic diabetic ulcers. Forty percent of ulcers treated with PBM were completely resolved within 8 weeks, with no reported adverse effects, whereas only 10% of control wounds healed completely within the 90-day study period. Group 2 and group 3 patients reported a significant decrease in ulcer discomfort and experienced occasional sharp pains which may be consistent with other clinical studies that have shown that PBM promoted an increase in nerve functional abilities and regeneration.	[71]
660	3 per spot	50	-	Irradiated daily for 15 days.	Patients with Type 2 DM with Meggitt- Wagner grade I ulcers of at least 6 weeks' duration. Patients were divided into two groups: PBM and control. All wounds received standard care involving debridement, slough excision and betadine solution dressings. Prior to PBM wounds were cleaned and gauze dried. Antibiotics were administered if necessary.	Visual examination of wounds and digital photography (wound area and wound contraction).	Wound size in both groups decreased over the 15-day period, with more healing noted in PBM treated wounds. Ulcers of the PBM group had more granulation tissue, while ulcers in the control group had more visible pus. PBM treated ulcers showed a significant reduction in wound area (37.3 ± 9%) as compared to control groups (15 ± 5%), and approximately 75% of these PBM treated wounds showed a reduction of 30-50% within the 15 days, compared to <20% in approximately 80% of control wounds.	[72]

Table 1. *Cont.*

Wavelength (nm)	Dose (J/cm^2)	Power Density (mW/cm^2)	Frequency (Pulse Duration)	Treatment Schedule	Wound Model	Outcome Measures	Outcomes/Observations	Reference
685	10	50	-	Irradiated six times per week for 2 weeks, and then every other day until healed.	Randomized double-blind controlled clinical trial and treated stage I and II diabetic foot ulcers. Patients were divided into two groups: PBM and control (placebo). All ulcers received standard care (debridement, off-loading, dressings and antibiotics).	Digital photography, peripheral neuropathy, and arterial ultrasound if deemed necessary.	Two and 4 weeks into the study, the decline in ulcer size in PBM treated ulcers compared with the baseline was significantly greater than in the placebo group (58 ± 10.4% vs. 23.5 ± 14.1% at 2 weeks and (73.7 ± 10.2% vs. 47.3 ± 15.4%, respectively). By 20 weeks, a larger number of PBM treated ulcers displayed complete healing, and the mean time of healing was lower (results were however insignificant).	[73]
Multidiode cluster probe (wavelength/s not provided)	2–4	(60 mW)	5 kHz	Irradiated daily for 15 days.	Type 2 DM patients with Meggitt- Wagner grade I ulcers of at least 4 weeks' duration. Patients were divided into two groups: PBM and control (placebo). Ulcers were subjected to debridement, off-loading and antibiotics administered if necessary. Ulcers were dressed daily with wet saline or betadine.	Ulcer area/percent reduction in the size.	At the end of the study period (15 days), there was no significant difference between PBM and control group with respect to wound area (1564.79 mm^2 for PBM and 2424.75 mm^2 for control group). The mean reduction in ulcer area was significantly more in the PBM group than in the control group (322.44 ± 85.84 mm^2 and 1043.20 ± 266.62 mm^2, respectively). Ulcers treated with PBM showed a significant reduction in percentage wound area (40.24 ± 6.30 mm^2) compared to 11.87 ± 4.28 mm2 in control groups	[74]
660	2	(100 mW)	-	Irradiated daily for 12 consecutive days.	Diabetic patients presenting with presenting grade II, III, or IV.	Digital photography and removal of granulation tissue for gene analysis (RT-qPCR)-IL-6, TNF-α, VEGF, and TGF-β.	PBM produced a reduction in wound size accompanied by an improvement of the biochemical markers: VEGF and TGF-β expression increased, and TNF-α expression decreased post-PBM. Wound size improved by around 50% after 7 days, and exhibited increased cellular activity at the wound edge and base, and faster formation of granulation tissue.	[75]

* Actual dose differed from incident dose due to losses due to the acrylic glass cage and reflection of light energy by the rat's skin. The actual doses were calculated and can be found in the original manuscript [49]. bFGF, basic fibroblast growth factor; CFU, colony forming units; EGF, epidermal growth factor; FBS, fetal bovine serum; FGF, fibroblast growth factor; IL-6, interleukin-6; IL-1β, interleukin 1 beta; LED, light emitting diode; MMP, matrix metalloproteinase; NO, nitric oxide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; p-EGFR, phosphorylated epidermal growth factor receptor; p-JAK2, phosphorylated janus knase 2; p-STAT1, phosphorylated signal transducer and activator of transcription 1; p-STAT5, phosphorylated signal transducer and activator of transcription 5; PDGF, platelet-derived growth factor; PMNL, polymorphonuclear leukocyte; ROS, reactive oxygen species; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; TIMP, tissue inhibitor of metalloproteinases; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

5. Conclusions

Wound repair is an attempt of the biological system to restore tissue integrity and denotes the outcome of numerous sequential, time-based, interconnected biological events that are highly coordinated in response to injury and its microenvironment. The colossal economic and social impact of chronic wounds on modern day society calls for a higher level of consideration and improved treatments. PBM has shown promising results in vitro, as well as in animal and human studies. The effects are reported to be anti-inflammatory; stimulate cell proliferation, viability, and migration; promote essential cytokine and growth factor production and release; foster collagen and ECM production; decrease inhibitory MMPs; and decrease oxidative stress, all vital processes to promote healing of chronic wounds and diabetic ulcers. Blue light has also been shown to be effective against the killing and inhibition of pathogens, including MRSA, providing promise for this therapy to be used in the treatment of infected wounds. Noninvasive, economical, and versatile light devices are an appealing tool for wound management, either in combination with standard care or alone, especially considering all of the cellular effects of PBM. This review provided a background for applications of PBM for wound healing under conditions of hyperglycemia. The shortage of rigorous, well-designed clinical trials makes it challenging to assess the scientific impact of PBM on DFUs, and lack of understanding of the underlying mechanisms also hinders the conventional use of this therapy. There is a crucial need for the wound care community to develop optimal clinical protocols for use based on well-designed studies, and for basic research to determine the underlying cellular and molecular effects, and mechanisms of action. Further research that makes use of the correct study design and laser parameters is required, and more studies on infected wounds are essential.

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