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REVIEW ARTICLE

Noninvasive red and near-infrared wavelength-induced photobiomodulation: promoting impaired cutaneous wound healing

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SUMMARY

The innumerable intricacies associated with chronic wounds have made the development of new painless, noninvasive, biophysical therapeutic interventions as the focus of current biomedical research. Red and near-infrared light-induced photobiomodulation therapy appears to emerge as a promising drug-free approach for promoting wound healing, reduction in inflammation, pain and restoration of function owing to penetration power in conjunction with their ability to positively modulate the biochemical and molecular responses. This review will describe the physical properties of red and near-infrared light and their interaction with skin and highlight their efficacy of wound repair and regeneration. Near-infrared (800–830 nm) was found to be the most effective and widely studied wavelength range followed by red (630–680 nm) and 904 nm superpulsed light exhibiting beneficial photobiomodulatory effects on impaired dermal wound healing.

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Cutaneous wound healing is a normal physiological process encompassing a series of well-orchestrated events culminating in the restoration of injured tissue. Healing proceeds through four overlapping phases: haemostasis, inflammation, granulation tissue formation and tissue remodelling. This simple process becomes multifaceted under certain pathophysiological conditions such as diabetes, venous stasis, pressure ulcers, burns, immunosuppression, which subsequently defers the process of tissue repair. Although the reasons behind delayed wound healing could be manifold, the major ones are prolonged inflammation, hypoxia, sepsis, compromised blood circulation, enhanced proteolysis, impaired expression of some growth factors, bacterial colonization/biofilms, excessive exudate and phenotypic changes in resident wound cells (1). Chronic nonhealing wound is one of the major therapeutic and economic issues in medicine today, which causes a great deal of physical and psychological discomfort and morbidity to affected patients. The chronic wound care accounts for an estimated cost

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of $15 billion annually in the United States. The precise costs remain unidentified due to difficulties in obtaining exact measurements because patients are seen in a variety of settings or even fail to access the healthcare system (2). In view of the aforementioned information, efforts are constantly in process to search newer and better cost-effective modalities of wound care to reduce the discomfort, morbidity and financial burden incurred to the patients.

Recent advances in cellular and molecular biology have greatly expanded our understanding of the biologic processes involved in tissue repair and have led to improvements in wound care management. Research on different pharmacological and nonpharmacological modalities to augment wound healing is a developing area in biomedical sciences. Currently, efforts are being made to explore novel strategies, viz. pharmacotherapeutic agents, bioactive dressings, tissue engineered scaffolds, stem cell-based therapy as well as drugless, noninvasive, biophysical therapeutic interventions using light-based treatment (photobiomodulation, PBM, or low-level light therapy, LLLT) (3), pulsed electromagnetic field (PEMF) modalities and bio-electrical stimulation, which can promote healing and thereby reduce the cost of hospitalization and save patients from amputation and other severe complications. This article will specifically address the recent advances in light-based therapy also known as PBM using medical red and near-infrared (NIR) light, and its applications in promoting dermal wound healing and subsequent future prospects.

PHOTOBIOMODULATION THERAPY

PBM has been defined as a form of light therapy that uses coherent light sources (lasers), noncoherent light sources consisting of filtered lamps or light emitting diodes (LEDs) or occasionally a combination of both, in the visible and NIR spectrum. It is a nonthermal process involving endogenous chromophores eliciting photophysical (i.e., linear and nonlinear) and photochemical events at various biological scales (4). The creative use of light-based therapies in the treatment of diverse pathophysiological conditions after its introduction as a therapeutic modality by Endre Mester in 1967 has gained major attention of the researchers worldwide in the past few decades. Since then, the field of PBM has been advancing constantly and widely used for the treatment of a variety of conditions, including healing of wounds, muscle and nerve injuries (3, 5, 6), reduction in inflammation and pain (7), restoration of function and photorejuvenation (8). Light-based treatment can be used in two completely different but complementary therapeutic applications, that is, PBM therapy and photodynamic therapy (PDT). PBM differs from other light-based treatments, as it does not ablate and does not rely on heating. It also differs from PDT, which is based on the effects of light to excite endogenous chromophores or exogenously delivered nontoxic photosensitizers and react with ambient oxygen to produce reactive oxygen species (ROS) that can kill infectious micro-organisms and cancer cells or destroy unwanted tissues (neovascularization in the choroids and altherosclerotic plaques in the arteries) (3). Eradication of micro-organisms without causing them to develop resistance coupled with the delivery of painless treatment by the light-based therapies gives rise to newer hopes for chronic wound healing.

INTERACTION OF LIGHT WITH SKIN

The skin functions as a barrier to the external environment to maintain fluid homoeostasis and body temperature while providing sensory information along with metabolic and immunological support (9). The body becomes prone to microbial infection as soon as the first line of defence, that is, skin undergoes disruption. It is an intricate heterogeneous structure which is naturally exposed to light more than any other organ and still responds well to red and NIR light delivered at the appropriate optical parameters with therapeutic intent (8, 10). The ability of light to penetrate a tissue and deposit energy via the optical absorption properties of the tissue is key to therapeutic applications (11). Even though light–tissue interaction mainly depends on the wavelength, it also gets affected by the tissue ingredients interacting with the photons. In tissue, an 'optical window' lies between 600 and 1350 nm where the effective tissue penetration of light is maximized due to minimum light attenuation owing to slight absorption and scattering by the major tissue components, viz. haemproteins, melanin, water, collagen. (10). Light possessing lesser or greater wavelength than this region is absorbed by the tissue components (haemoglobin, myoglobin in visible region and tissue water content in the IR region). Moreover, biological tissue has lower scattering capacity at NIR region than at visible region. This implies that within this range, biological experiments can be performed without damaging the skin tissue, because it involves lesser light–tissue interaction (12). The infrared (IR) spectral region accounts for approximately 40% of the solar radiation encompassing three prominent bands:

IR-A (760–1400 nm), IR-B (1400–3000 nm) and IR-C (3000 nm-1 mm) (13). Among these, only the IR-A (30% of the IR radiation) fraction reaches the human body and penetrates deeply into the skin, and 65% of that portion reaches the dermis, thereby making IR-A well positioned to exert bio-effects on the dermis (14). The fact that red and NIR (IR-A) light can penetrate into a deep dermal tissue injury allows nonpharmacological, noninvasive treatment to be carried out for augmented healing processes (15).

The effect of optical radiation on biological systems primarily depends on two things: the amount of energy absorbed and the rate of deposition of energy. The energy absorbed per unit volume of the tissue will be proportional to the product of the radiation intensity, its pulse duration and the absorption coefficient of the tissue at the wavelength of the radiation. In PBM, successful therapeutic outcomes require selection of optimum optical treatment protocols including illumination parameters (such as wavelength, fluence, power density, pulse structure) and the treatment schedule. Optical dosages, that is, delivered fluence (J/cm^2) and irradiance (mW/cm^2) that are either substantially less or substantially more than optimal choice of optical parameters can result in reduced effectiveness of the treatment or even a negative therapeutic outcome (16). Indeed, the existence of the biphasic dose–response has been held responsible for the publication of a number of negative studies, where it is highly likely that the results would have been positive if different parameters had been employed (15, 17).

Both scattering and absorption of light by tissue are highly wavelength dependent, and NIR light around 810–830 nm has been found to have the best penetration into tissue with number of proven cellular targets. It has been reported that visible red light is readily absorbed by blood and skin surface components, hence limiting its tissue penetration (<10 mm); however, NIR light is not readily absorbed and has much greater depth of tissue penetration (>30–40 mm), thereby providing greater deposition of photons in the wound bed, and showed therapeutic healing efficacy (18). Several studies have shown the beneficial effects of NIR 810 nm light for treatment of different kind of injuries. The PBM of 810 nm irradiation improves neurological performance in traumatic brain injury (19, 20), accelerates both normal and chronic dermal wound healing (3, 21, 22) and prevents the appearance of surgical scar (23).

The mode of operation of PBM therapy can be either continuous or pulsed wave (CW/PW). The recent studies continue to demonstrate that PW does have biological and clinical effects that are different from those of CW. It has been reported that PW is more effective than CW, as there are quench periods (pulse-off times) of longer duration than the on-timings which reduce tissue heating. Several studies revealed that the LLLT in PW mode of operation can better penetrate through the melanin and other skin barriers, supporting the hypotheses that pulsing is beneficial in reaching deep target tissue and organs (16, 19). Some recent studies exhibited PW mode to be beneficial over CW mode, especially in the context of wound repair and stroke management (16).

Superpulsed LLLT emits tremendously short pulses in the order of billionth of a second (10^{-9} s), and this unique feature entails it with a predominant mechanistic advantage of administering extremely high-peak powers followed by the accumulation of more energy in the tissue undergoing healing process. These extremely small pulses allow quick absorption at the cellular level, and the period between pulses promotes a better environment for enhanced cell communication leading to an optimum form of augmented healing. These numerous benefits of superpulsed (904 nm) LLLT have demonstrated therapeutic potential, particularly in the context of impaired wound healing (18). The intermittent nanosecond pulsing patterns in superpulsed LLLT might enhance multiple photodissociation events of NO from CCO, which in turn enhances mitochondrial respiration. The penetration power of continuous wave and superpulsed wave through different layers of skin has been

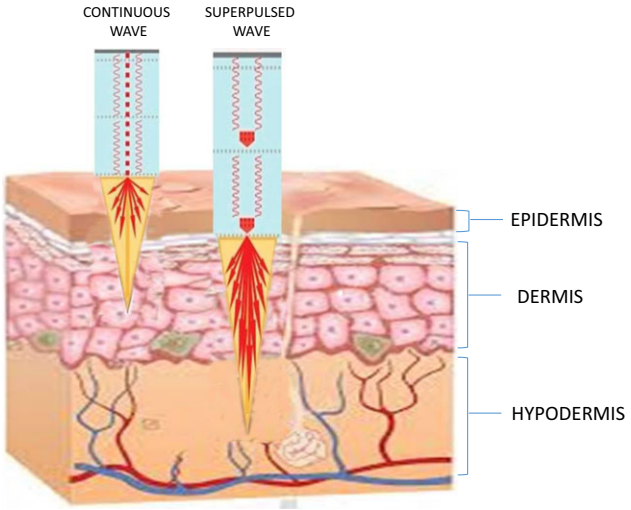

Fig. 1. Tissue penetration depth of continuous and superpulsed wave. The intensity of superpulsed mode is much higher than continuous mode which leads to profound penetration, thereby providing the ability to heal deep-seated dermal injuries.

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Table 1. Summary of photobiomodulatory effects of red and near-infrared (NIR) light in promoting dermal wound healing

Target	Laser/LED type Optical parameters	Wave mode	Preclinical /Clinical	Salient findings	Ref.
Diabetic wound healing in rats	OLED (623 nm wavelength peak, range from 560 to 770 nm, 7 or 10 mW/cm^2, 0.2, 1 or 5 J/cm^2); daily exposure for 7 days	Not mentioned	*In vivo*	OLED and laser had comparative effects on wound healing	Wu *et al.*, 2015 (28)
Second-degree burn wound healing in rats	Red (660 nm, 100 mW, 20 J/cm^2); daily exposure starting from day 1 in one group and from day 4 post injury in another group for 5 days	Continuous	*In vivo*	Red laser contributes to tissue repair process, and the intervention is crucial during proliferative phase	Trajano *et al.*, 2015 (29)
Sutured skin incisions in porcine model	Red (685 nm, 0.008 mW/cm^2, 3.36 J/cm^2) and blue (470 nm, 0.008 mW/cm^2, 3.36 J/cm^2); daily exposure for 3 and 7 days in two different groups.	Not mentioned	*In vivo*	Combined red and blue photobiomodulation accelerated the process of re-epithelialization and formation of cross-linked collagen fibres.	Figurova *et al.*, 2016 (30)
Skin wounds in Japanese big-ear white rabbit	Red LED (630 nm, 50 mW/cm^2, 45 J/cm^2), blue (460 nm, 50 mW/cm^2, 90 J/cm^2) LED; daily exposure of 15 and 30 min in the 2 irradiated groups	Not mentioned	*In vivo*	Red light hasten wound healing by promoting fibrous tissue, epidermal and endothelial cell proliferation	Li *et al.*, 2016 (31)
Burn tissue repair in mice	Red (632.8 nm) and near-infrared (785 and 830 nm), 8.49 mW/cm^2 (1, 2, 3, 4 and 6 J/cm^2); single exposure	Not mentioned	*In vivo*	Single exposure of 830 nm with fluence 3 J/cm^2 enhanced burn wound healing in mice which is equivalent to 5% povidone–iodine applied daily till complete healing	Rathnakar *et al.*, 2016 (32)
Cutaneous wound healing in rats	635 nm, 1 and 3 J/cm^2;	Not mentioned	*In vivo*	Laser irradiation of lower energy density more effective during the first days of healing process	Solmaz *et al.*, 2016 (33)

Table 1. Continued.

Target	Laser/LED type Optical parameters	Wave mode	Preclinical /Clinical	Salient findings	Ref.
Cutaneous healing in rats	660 nm, 40 mW, 6 J/cm^2; irradiation started immediately after surgery and every 48 h for 7 or 14 days	Continuous	*In vivo*	Positive biomodulative effect on the healing process in early stages	Uzeda-E-Silva *et al.*, 2016 (34)
Full-thickness burn wound in rats	904 nm, 100 Hz, 0.4 mW/cm^2, 0.2 J/cm^2; daily exposure for 7 days	Superpulsed	*In vivo*	Attenuated inflammation and augmented healing in rats	Gupta *et al.*, 2015 (35)
Diabetic wounds in mice	904 nm, 9500 Hz, 304.8 mW/cm^2, 18.288 J/cm^2; daily irradiation for 5 days	Superpulsed	*In vivo*	Increased collagen production and decreased oxidative and nitrosative stress during diabetic wound healing	Rocha *et al.*, 2016 (36)
Diabetic wounds in mice	830 nm, 4.2 J/cm^2, 14 mW/cm^2; low-level light therapy (LLLT) was applied every 2 days starting in 24 h of wound formation	Continuous	*In vivo*	Higher collagen levels (50%), additive enhancement of ATP and threefold increased cell proliferation in LLLT- and CoQ10-treated wounds over nonirradiated controls	Mao *et al.*, 2016 (37)
Diabetic ulcer for burn patients (type 3)	650 nm, 2 J/cm^2 810 nm, 6 J/cm^2; irradiation given before and after STSG	Continuous	Clinical	Diabetic ulcer with type-3 burn wound healed completely using LLLT and split-thickness skin grafting	Dahmardehei *et al.*, 2016 (38)
Partial-thickness dermal abrasion in mice	(635, 730, 810 and 980 nm), 4 J/cm^2, 10 mW/cm^2; daily for 7 days	Continuous	*In vivo*	Maximally augmented healing observed in 810-nm laser-treated group	Gupta *et al.*, 2014 (18)
Second-degree burn in rats	(670 nm) (810 nm); 2, 6, 10, 14, and 18 days of treatment	Continuous	*In vivo*	Different types of light sources showed similar effects, improved the healing of second-degree burns	Chiarotto *et al.*, 2014 (39)

Table 1. Continued.

Target	Laser/LED type Optical parameters	Wave mode	Preclinical /Clinical	Salient findings	Ref.
Full-thickness dorsal excisional wound in mice	635 nm, 1 and 2 J/cm^2, 2 and 1 mW/cm^2; 670 nm, 1 J/cm^2, 0.59 mW/cm^2; 720 nm, 1 J/cm^2, 0.79 mW/cm^2; 820 nm, 1 J/cm^2, 0.86 mW/cm^2; single exposure 30 min postwounding	Continuous	*In vivo*	820 nm wavelength was found to be most effective	Demidova-Rice *et al.*, 2007 (40)
Human skin	640 nm, 2 Hz, 16.66 mW/cm^2 + 875 nm, 16 Hz, 19.44 mW/cm^2; 905 nm, 250 Hz, 0.71 mW/cm^2	Pulsed and superpulsed	Clinical	Concurrent use of superpulsed lasers, pulsed red and infrared LEDs can be utilized in patients with all types of skin pigmentation without concern over safety or excessive tissue heating	Grandinetti *et al.*, 2015 (41)
Burn scars	1550 nm; irradiation given at 4-week intervals	Continuous	Clinical	Skin texture, dyschromia and hypertrophy improved in 90%, 80% and 80%, respectively	Waibel *et al.*, 2012 (42)
Second-degree burn in rats	890 nm, 3000 Hz, (2.3 J/cm^2 and 11.7 J/cm^2)	Pulsed	*In vivo*	Pulsed LLLT with 11.7 J/cm^2/ 890 nm of a deep second-degree burn model in rat significantly increased the rate of wound closure compared with control burns	Ezzati *et al.*, 2010 (43)
Third-degree burn in rats	890 nm, 3000 Hz, (2.3 J/cm^2 and 11.7 J/cm^2); irradiation was given three times a week	Pulsed	*In vivo*	Pulsed LLLT with 11.7 J/cm^2/ 890 nm of a third-degree burn in a rat model significantly increased wound closure rate compared with control burns	Ezzati *et al.*, 2009 (44)
Partial-thickness wounds	820 nm (2.5, 20, 292, 20 000 Hz), 800 mW/cm^2, 21.6 J/cm^2	Pulsed	*In vivo*	Degranulation is pulse frequency dependent, whereas mast cell number is not.	El Sayed *et al.*, 1996 (45)

Table 1. Continued.

Target	Laser/LED type Optical parameters	Wave mode	Preclinical /Clinical	Salient findings	Ref.
Superficial wounds	820 nm, 700 Hz, 8 J/cm^2; daily treatment for 10 days	Pulsed	Clinical	LLLT is an effective modality to facilitate wound contraction of partial-thickness wounds	Hopkins *et al.*, 2004 (46)
Surgical scar	810 nm	Continuous	Clinical	Appearance of a surgical scar improved by 810-nm diode laser treatment	Capon *et al.*, 2010 (23)
Full-thickness skin lesions	830 nm, 53 mW/cm^2, 1.3 J/cm^2 and 3 J/cm^2	Continuous	*In vivo*	Faster lesion contraction observed in 1.3 J/cm^2 dose-treated group	Rezende *et al.*, 2007 (21)
Wounds in diabetic rats	810 nm, 30 mW/cm^2, 0.9 J/cm^2; daily treatment	Continuous	*In vivo*	LLLT improved wound healing in rats	Dancakova *et al.*, 2014 (22)

shown in Fig. 1. Studies have reported that superpulsing is playing an important role in PBM especially for clinical application where deep tissue penetration (up to 10 cm, for bones, tendons, ligaments and cartilages) is required (24). Table 1 is a summary of studies concerned with photobiomodulatory effects of red and NIR light-induced dermal wound healing.

MECHANISMS FOR PHOTOBIOMODULATION

Studies have reported that the photobiomodulatory effects are induced by athermic physiological processes producing photochemical and photobiological changes at cellular and molecular levels (3, 25). The cellular and molecular mechanisms of action of PBM have become reasonably well understood in recent years and are summarized in Fig. 2. Briefly, the stimulatory effect of PBM is based on the absorption of red and NIR photons by intracellular chromophores located within the mitochondria (cytochrome c oxidase, CCO), and perhaps also by chromophores in the plasma membrane of cells, altering the activity of one or more endogenous enzymes and electron transport, thereby increasing mitochondrial respiration and ATP production (3, 26). Moreover, the biological effectiveness of wavelengths that are much longer than those absorbed by CCO and few other effects that are not explained by overstimulation of mitochondria has been proposed to be occurring through the biological influence of PBM on ion channels (27). Light-sensitive ion channels can be activated allowing Ca^{+2} to enter the cell and eventually diverse intracellular signalling pathway activation mediated by ROS, nitric oxide (NO), cyclic AMP (cAMP) and Ca^{+2} leading to the activation of transcription factors concerned with protein synthesis, extracellular matrix (ECM) deposition, cell migration, proliferation, anti-inflammatory, cell survival and inhibition of apoptosis. Stem cells and progenitor cells seem to be particularly susceptible to PBM therapy (3, 26). CCO has distinct absorption bands in the red (around 665 nm) and in the NIR (around 810 nm) regions (25). Furthermore, it has been suggested that nonthermal irradiation forms of light in the visible or NIR spectral range activate retrograde light-sensitive cellular signalling events to transport the light signal from mitochondria to the nucleus and alter the cell metabolism and functions (25). Besides CCO, a number of other molecules have been proposed to act as photoacceptor, depending on the wavelength of excitation. Flavins and flavoproteins, free porphyrins, haemoglobin and myoglobin, and 'nanostructured water' (in the IR spectral region) have all been proposed to be able to absorb

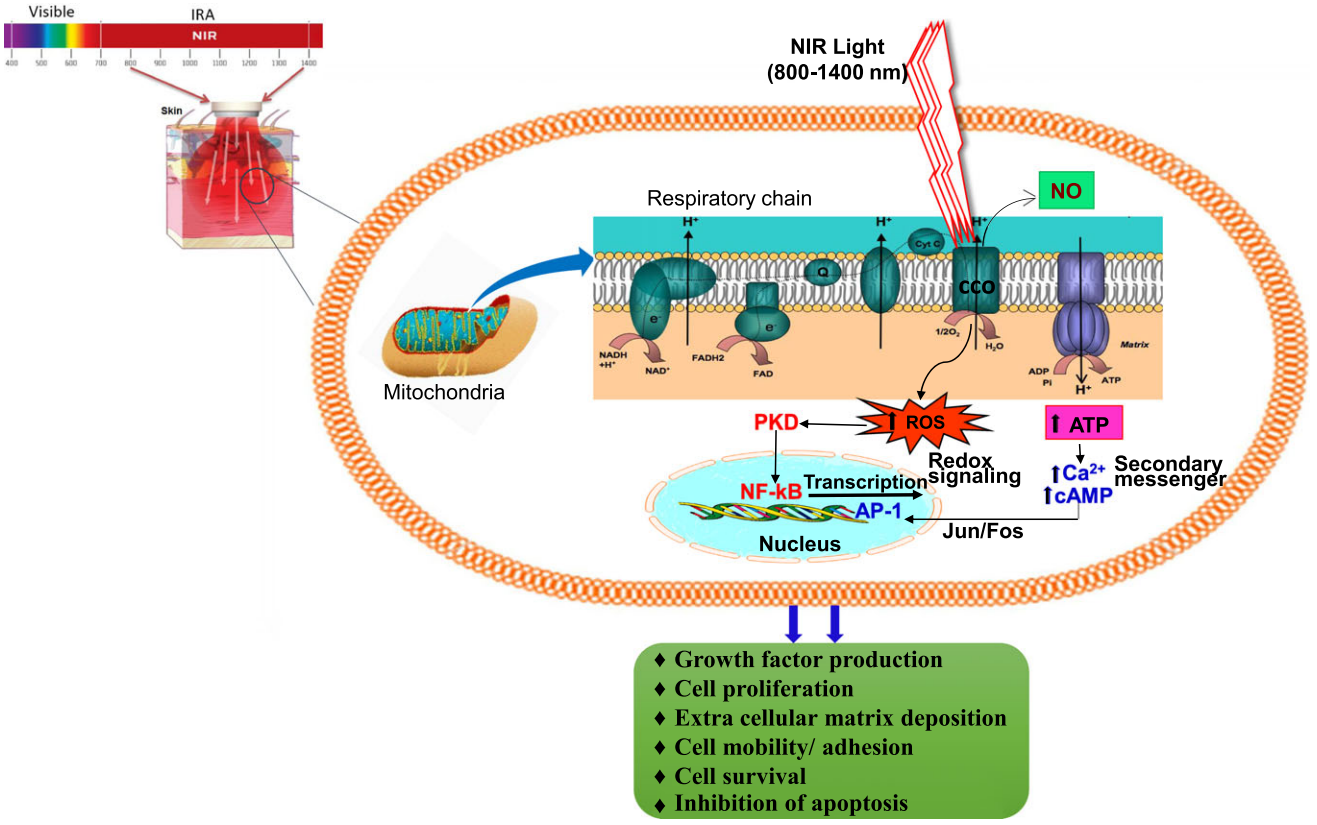

Fig. 2. Solar spectrum depicting visible and near-infrared (NIR) light regions and skin penetration depth of NIR light. Mechanisms of action of photobiomodulation (PBM), NIR light initially absorbed by mitochondrial chromophore (photoacceptor, cytochrome c oxidase, CCO). Photon absorption leads to dissociation of inhibitory nitric oxide (NO) from CCO leading to increased enzyme activity and raised ATP production and a burst of reactive oxygen species (ROS), which in turn cause changes in cellular redox potential, Ca2+, cAMP and induce several transcription factors (NF-kB, AP-1, etc.). Photosignal transduction and amplification chain induced by NIR light lead to an increase in growth factor production, cell proliferation, cellular mobility, adhesion and extracellular matrix deposition. (cAMP – cyclic adenosine monophosphate; NF-kB – nuclear factor-kB; AP-1 – activating protein-1; ROS – reactive oxygen species; PKD – protein kinase D; NO – nitric oxide).

photons and mediate photochemical and photobiological reactions. The establishment of a well-defined mechanistic approach towards PBM will help in unravelling the downstream pathways which can boost future research endeavours and clinical applications of red and NIR light in dermal wound repair.

LIMITATIONS OF PHOTOBIOMODULATION THERAPY

PBM therapy has been practiced for more than five decades, and several positive clinical trials and laboratory studies have been reported. Despite impressive therapeutic benefits, PBM has still not achieved the stage of acceptance by mainstream medicine. The major limitations in this research field lie in two areas: first, uncertainty about the cellular and molecular mechanisms responsible for transducing signals from the photons incident on the cells to exert the bio-effects that take place in the irradiated tissues. Second, there are a large number of optical dosimetry parameters. These parameters are required to be standardized for each anomaly as there is more variation in the parameters of PBM for the treatment of the same type of injury. To overcome these limitations, recently researchers have given major emphasis on the understanding of substantial mechanistic insights, better guidelines with standardized protocols, consistency in radiant exposure parameters and systematically controlled clinical studies.

CONCLUSION

In aggregate, red and NIR light-induced PBM can be considered as a promising biophysical healing modality owing to its numerous applications. Standardization of optical parameters is the major pointing issue with this

technique which varies for different maladies. Recently, NIR light has gained attention as a potential treatment strategy for both the acute and chronic dermal wound repair and regeneration. This article emphasized further research into the biological role of red and NIR lights on the dermal wound repair. NIR light penetrates more deeply into the skin, absorbed by distinct set of cellular chromophores, activates a unique set of molecular target, which in turn promotes repair, regeneration and cell survival, prevents apoptotic cell death and consequently processes the bio-effects on the dermal tissue that are different from other forms of spectral radiant energy. The fast growing field of NIR light-induced PBM will continue to offer painless, potential noninvasive, drugless biophysical therapeutic intervention for chronic nonhealing dermal wounds, especially when conventional therapies have failed or have unaccepted side effects. The development of such noninvasive, light-based healing therapies appears to be much needed to stay away from the threat of antibiotic resistance and the unwelcome side effects caused by pharmaceuticals.

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