

PHOTO-BIOMODULATION-INDUCED OXIDATIVE STRESS IN CHRONIC WOUND PATHOGENS: OPTIMIZING LASER PARAMETERS FOR BACTERIAL ERADICATION AND FIBROBLAST PROLIFERATION

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ABSTRACT

Chronic wounds colonised by antibiotic-tolerant biofilms represent a growing clinical and economic burden, and light-based therapies have emerged as a promising adjunct that exploits oxidative stress for therapeutic ends. This paper presents a qualitative evidence synthesis of the experimental literature on photobiomodulation (PBM) and related light therapies, examining how irradiation generates oxidative stress in wound pathogens and host cells and how laser parameters might be optimised to achieve the dual goal of bacterial eradication and fibroblast proliferation. Following an integrative review approach, peer-reviewed studies were identified, appraised, and analysed using reflexive thematic synthesis. Six themes were constructed: the mechanistic basis of light-induced oxidative stress; the antimicrobial dimension of short-wavelength light and reactive oxygen species (ROS); the regenerative dimension of red and near-infrared light on fibroblasts; the biphasic dose-response and the therapeutic window; the optimisation of wavelength, fluence, irradiance, and delivery; and translational barriers including biofilms, penetration, and safety. The synthesis indicates a fundamental duality: high, sustained ROS production, achieved chiefly with blue light exciting endogenous porphyrins, drives microbial death, whereas brief, sub-lethal ROS bursts produced by red and near-infrared light activate redox signalling that stimulates fibroblast proliferation and collagen synthesis. These opposing outcomes are governed by the same Arndt-Schulz biphasic principle, implying that wavelength and dose must be selected to maximise the gap between microbial kill and host-cell stimulation. The review concludes that no single parameter set is universally optimal; rather, sequential or combined wavelength strategies, calibrated to wound bioburden and tissue depth, offer the most coherent path toward simultaneous disinfection and regeneration, and it identifies standardisation of dosimetry as the principal research priority.

KEYWORDS: photobiomodulation, oxidative stress, reactive oxygen species, chronic wounds, antimicrobial blue light, fibroblast proliferation, laser parameters

INTRODUCTION

Chronic wounds, such as diabetic foot ulcers, venous leg ulcers and pressure ulcers, are a big and growing burden on health systems globally, using a disproportionate amount of wound care resources and reducing the quality of life (QoL) of the patient (Sen et al., 2009). One of the main causes of these wounds not healing is a continuous microbial colonization. Most chronic wounds contain structured populations of bacteria (aka biofilms) in which bacteria like *S. aureus* and *P. aeruginosa* are protected from host defenses and antibiotics by their own produced matrix, resulting in significantly higher tolerance (James et al., 2008; Malone et al., 2017). Systemic antibiotics often fail to eradicate biofilm-associated infection and promote the global issue of antimicrobial resistance.

The magnitude of the problem makes it urgent to look for alternatives. The prevalence of chronic wounds has increased significantly with the global rise of diabetes, obesity and ageing, and chronic wounds contribute significantly to health care expenditure on wound care, hospitalisation and in severe cases of diabetic foot disease, amputation (Sen et al., 2009). Meanwhile, the number of new antibiotics coming online has declined while antibiotic resistant organisms like methicillin-resistant *Staphylococcus aureus* (MRSA) have become regular wound isolates. Ideally, a wound treatment should decrease the microbial load without inducing resistance, and should stimulate wound healing, rather than simply limiting microbial proliferation. Under these circumstances, the oxidative mechanisms of Light Therapy seem to be attractive since it is believed that bacteria are difficult to withstand a lethal mechanism involving oxidative damage to multiple targets (Dai et al., 2012).

These pressures have led to a focus on alternative therapies that involve the use of light rather than traditional antibiotics. There are two closely related, but different modalities. Antimicrobial blue light and antimicrobial photodynamic approaches, the first, involve the deliberate application of oxidative stress to the pathogens, by short-wavelength light excitation of endogenous or exogenous photosensitisers to generate reactive oxygen

species (ROS) that damage microbial lipids, proteins and nucleic acids (Dai et al., 2012; Wang et al., 2016). The second, or low level laser therapy (LLLT), as it was previously known (now called photobiomodulation, or PBM), is a therapy that utilizes red and near infrared light, in lower doses, to stimulate tissue repair, such as the proliferation and migration of fibroblasts, which rebuild the dermal matrix (Avci et al., 2013; Hawkins & Abrahamse, 2006). Strikingly, both modalities are mediated by oxidative chemistry. The difference is in the amount, length and context of cells of ROS.

Some remarks on terminology are in order, as the field's terminology has also evolved over time. The non-thermal use of red and near-infrared light for modulation of biological activity has been generically referred to as low-level laser therapy (LLLT) or low-level light therapy (LLLT), but a consensus has emerged to use the term "photobiomodulation" to indicate the use of light to modulate biological activity without causing thermal or photoablative effects, especially to distinguish it from the use of higher power lasers for therapeutic photothermal and photoablative effects (Anders et al., 2015). In this paper, the term "photobiomodulation" is used to describe the re-generative and biostimulatory applications of light, whereas the applications of antimicrobial blue light and antimicrobial photodynamic therapy are used to describe the applications that are meant to be cytotoxic, with the production of ROS. The frames used throughout are based on the premise that these are not separate methods, but steps along a shared oxidative continuum in terms of wavelength and dose.

This common reliance on oxidative stress is shared and gives rise to an opportunity and tension that is the frame for the present review. Heroism is that theoretically, a single physical agent – light – can disinfect a wound and close it at the same time, without the disadvantages of resistance that would come with the use of antibiotics. The challenge is that the oxidative stress needed for the elimination of bacteria, if it is too strong or not targeted at the bacteria, can also have a negative effect on the host cells that are supposed to multiply. This synthesis is concerned with the question of whether and how laser parameters can be optimised to eliminate the tension between killing the pathogens while preserving and even stimulating the fibroblasts.

This paper takes a qualitative evidence-synthesis approach, as the topic is inherently experimental, and evidence is spread across the fields of photobiology, microbiology and wound science. It thematically brings together the published experimental literature and builds up a coherent picture of mechanisms and parameters. The goals are three-fold: to elucidate the differential effects of light-induced oxidative stress on pathogens and host cells; to synthesize the information from the literature to conclude on optimal wavelengths, fluences and irradiance for the dual goal; and to define the translation gaps that need to be filled before optimal wavelengths, fluences and irradiances can be translated clinically. The rest of the paper outlines the review methodology, presents six synthesised themes and their implications for parameter selection and future research.

Design and Rationale

A qualitative evidence synthesis was used in this study, which was based on an integrative review with reflexive thematic synthesis. Integrative review was chosen because it allows for the synthesis of multiple empirical sources, in vitro, in vivo, and review literature, that fits a question that is multi-disciplinary (Whittemore & Knafl, 2005). Instead of adding together effect sizes as quantitative data, the results of the primary studies were treated as qualitative data and analysed using thematic synthesis, which has been developed for systematic reviews of complex evidence (Thomas & Harden 2008) and the reflexive thematic analysis framework of Braun and Clarke (2006).

Search Strategy and Sources

Key reviews were located in electronic databases (PubMed, Scopus, Web of Science) and reference-list screening, and the relevant peer-reviewed literature was identified. The search terms included elements of three fields: light therapy, oxidative chemistry, and wound biology. The search terms were a combination of concepts from three fields: light therapy (photobiomodulation, low-level laser therapy, antimicrobial blue light, photodynamic therapy), oxidative chemistry (reactive oxygen species, oxidative stress, singlet oxygen, porphyrin), and wound biology (chronic wound, biofilm, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, fibroblast, proliferation, collagen).

Selection and Appraisal

Sources were selected if they discussed either mechanisms or experimental results related to light-induced oxidative stress in wound pathogens or light effects on viability and proliferation of the fibroblasts and if they provided at least one of the following irradiation parameters: intensity, wavelength, time, or dose. Appraisal was qualitative for studies in terms of methodological transparency, specifically reporting wavelength, fluence, irradiance, and exposures conditions, as this is a known limitation in this area of study. Studies with clear and repeatable parameters received more weight than the others.

Synthesis Procedure

The six phases of reflexive thematic analysis (Braun & Clarke, 2006) were used for analysis. To familiarise the reviewer with the corpus he was first read, then made some findings and extracted descriptive codes inductively, that were attached with the support surface of the mechanistic claims, the values of the parameters, and the reported results. Codes were assembled into candidate themes and evaluated for coherence and distinctiveness with the entire corpus, and given names and definitions. All in all it was an explicit interpretive synthesis with the aim of developing a defensible conceptual framework of the relationships between parameters and the dual

therapeutic goal. Documentation of search and coding decisions was transparent, and all interpretive claims were backed up with citations of primary sources.

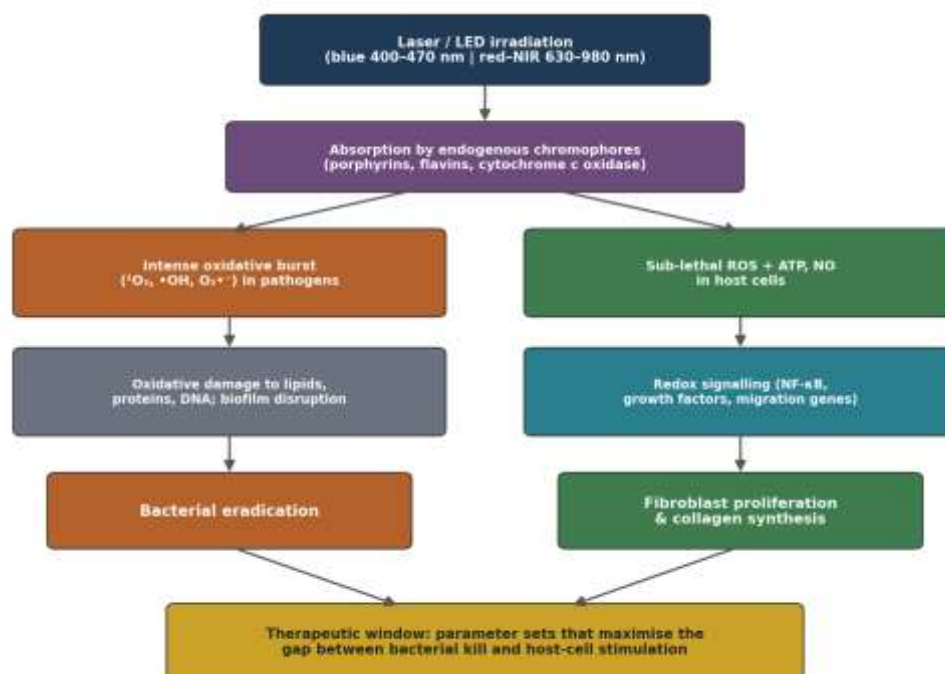
Synthesis of the Literature

Six themes were developed from the literature. They transition from the mechanical to the two contrasting therapeutic aspects, then to the dose principle, and the practical parameters it suggests and the obstacles to translation. The concept of these themes is represented in figure 1, showing the single oxidative pathway that may lead to bacterial eradication or to stimulation of fibroblasts depending on dose and context.

Theme 1: Mechanistic basis of light-induced oxidative stress

The literature comes together to a meaningful mechanistic explanation, in which light is absorbed by some kind of chromophores and is transformed into chemical and redox signals. In photobiomodulation, the most important chromophore is cytochrome c oxidase, the last enzyme in the mitochondrial respiratory chain, and light-sensitive ion channels are also thought to be involved (Hamblin, 2017; Karu, 1999). Secondary events of photon absorption involve a short-lived rise in adenosine triphosphate (ATP) production, nitric oxide (NO) production, and release of ROS (de Freitas & Hamblin, 2016). These secondary messengers then go on to activate transcription factors and downstream pathways that regulate survival, proliferation and migration. Hamblin (2018) highlighted the importance of mitochondrial redox signaling in this cascade and the fact that the ROS burst resulting from the red and near-infrared light is typically small and brief.

Figure 1. Conceptual framework of the dual action of light-induced oxidative stress in the wound.



Theme 1: Mechanistic basis of light-induced oxidative stress

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The antimicrobial effect of light has this same oxidative mechanism but targets a different chromophore and is more intense. The wavelengths used in antimicrobial blue light are within the range from 400 to 470 nm and contain wavelengths that activate endogenous photosensitisers present in bacteria, mainly iron-free porphyrins and flavins (Dai et al., 2012; Wang et al., 2016). These molecules can be excited, which initiates two types of photochemistry: Type I, which yields superoxide, hydrogen peroxide and hydroxyl radicals and Type II, which yields singlet oxygen. These species accumulate and overwhelm the microbes microbicidal arsenal and compromise critical biomolecules. An important lesson learned from all this work is that the porphyrins found in bacteria frequently have higher levels than the mammalian cells, and therefore are somewhat more selective in targeting microbial cells than mammalian cells (Hamblin et al., 2005). The mechanistic theme thus sets the stage

for the synthesis as a whole: oxidative stress is produced by a single physical agent, the chromophore involved, the wavelength used and the amount of ROS produced will influence whether signalling or destruction occurs. The two photochemical pathways described above are particularly important for antimicrobial production, the latter being the Type II production. Singlet oxygen is highly reactive, has a short life, and a short diffusion distance, is not easily reduced by the bacterial antioxidant enzymatic systems (such as superoxide dismutase and catalase) which are efficient at reducing superoxide and peroxide, respectively (Dai et al., 2012). The strength of this combination is augmented by the lack of a specific defence against oxidative killing, thus supporting the idea that oxidative killing is hard for bacteria to withstand. The intensity of the same chemistry is normally, however, engaged in a controlled burst in host cells, rather than indiscriminate injury, and Hamblin (2018) emphasized that the intensity and location of the ROS, along with the redox state of the cell, dictate whether the outcome is adaptive signaling or oxidative injury.

Hamblin (2017) categorized these events into a valuable hierarchy. Primary effects refer to the photophysical absorption of photons by chromophores; secondary effects are the immediate biochemical effects such as release of nitric oxide and modulation of intracellular calcium as well as the ROS burst; tertiary effects are the downstream activation of transcription factors and gene-expression programmes involved in proliferation, migration, antioxidant defence, and anti-inflammatory signalling. This staged model explains why the time and dose of the primary absorption is so important: If that same primary absorption is delivered at the wrong time and in the wrong amount, it can shift the secondary ROS response from a brief signal to continuous oxidative stress, and have opposite biological effects for a pathogen and for a fibroblast.

A more specific mechanistic suggestion relates to the way in which the regenerative cascade is initiated at cytochrome c oxidase by red and near-infrared light. One hypothesis, which has been discussed extensively, is that when under stress, NO can bind to the enzyme to stop respiration, but the bound NO is broken down by the absorbed light and respiration starts again, increasing the mitochondrial membrane potential and ATP production (de Freitas & Hamblin, 2016; Karu, 1999). The accompanying slight increase in ROS then acts as a second messenger, not a toxin. This account connects the photophysics directly with the downstream signalling and provides justification for the choice of red and near-infra-red wavelength for biostimulation as these penetrate tissue and are absorbed effectively by the appropriate redox centres. The coming and going of all of the hypothesis components is not what matters, though, but the fact that it embodies the core mechanistic intuition of the field—namely that low doses of light "untie" a metabolic brake and "light off" a controlled oxidative signal, while high doses or short wavelengths cause a "tie" that renders the same chemistry destructive.

Theme 2: The Antimicrobial Dimension

The aim of this Theme is to investigate the antimicrobial dimension of short wavelength light and ROS mediated killing. This Theme aims to examine the antimicrobial dimension of short wavelength light and ROS mediated killing.

A significant amount of experimental data have shown that the short wavelengths of light can destroy wound-relevant pathogens by oxidative damage. Wang et al. (2016) demonstrated that 415 nm antimicrobial blue light was effective to reduce bacterial burden in Gram-negative bacteria (e.g., *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) both in planktonic culture and in biofilms, with multi-log reductions in colony forming units observed in infected wounds in vivo. Mechanistic studies demonstrate that this killing is ROS-dependent; ROS suppression lessens lethality, and the damages observed are in the form of lipid peroxidation, protein carbonylation, and membrane permeabilisation (Wu et al., 2018).

The antimicrobial efficacy of light is strongly dose- and species-dependent. Killing increases with cumulative fluence, and the doses required for substantial microbial inactivation are considerably higher than those used for biostimulation; Wang et al. (2016), for example, reported biofilm inactivation at fluences on the order of several hundred joules per square centimetre. Susceptibility also varies by organism according to endogenous chromophore content and redox-defence capacity.

The antimicrobial reach of light extends beyond endogenous-porphyrin photoexcitation. Antimicrobial photodynamic therapy adds exogenous photosensitisers, such as methylene blue, toluidine blue, and porphyrin derivatives, which broaden the approach to organisms with sparse endogenous chromophores and allow activation with red light that penetrates more deeply than blue (Yin et al., 2013). Review evidence indicates light-dose-dependent killing of wound pathogens, including *Pseudomonas aeruginosa* and *Escherichia coli*, across animal wound and burn models, and the same work notes that potentiating agents can substantially increase killing efficiency (Yin et al., 2013). Susceptibility nonetheless varies by organism: differences in porphyrin content, cell-wall architecture, and antioxidant capacity mean that Gram-positive and Gram-negative species, and even strains within a species, are not equally vulnerable to a given exposure (Wu et al., 2018).

The antimicrobial effects of light are highly dose and species dependent. Killing increases with cumulative fluence and the dose needed to achieve significant microbial destruction is much greater than that needed to achieve microbial biostimulation, as reported by Wang et al., (2016) who found that a biofilm could be reduced to an inactive state at levels on the order of several hundred joules per square centimetre. Susceptibility also depends on the organism as a function of endogenous chromophore content and redox-defence capacity.

The photoexcitation of endogenous-porphyrins is not the sole mechanism for the microbial effect of light. APDT (APDT) introduces externally administered photosensitisers, like methylene blue, toluidine blue, and porphyrin derivatives, which extend the range of organisms that are susceptible and use a red light that can penetrate deeper than blue light (Yin et al., 2013). Studies have shown light-dose dependent killing of wound pathogens, such as

Pseudomonas aeruginosa and *Escherichia coli*, in animal models of wounds and burns, and potentiating agents can greatly enhance the killing efficiency (Yin et al., 2013). However, there is also inter-species variability in susceptibility, as porphyrin content, cell-wall structure and antioxidant activity vary between the Gram-positive and Gram-negative species and even between strains within a species (Wu et al., 2018).

Another area of the antimicrobial literature relates to synergy with traditional antimicrobial agents. Since oxidative light damage and antibiotic mechanisms are different, their combination can lead to additive or synergistic killing and may be useful to re-sensitize antibiotic tolerant biofilm organisms, which is a very attractive proposition by decreasing the reliance on antibiotics in wound care (Dai et al., 2012). The antimicrobial theme provides one side of the therapeutic puzzle: multiple light-based strategies exist for achieving effective, resistance-independent bacterial killing—although each requires relatively high doses of oxidative energy, thus raising the central issue of the tolerability of host tissue to oxidative stress.

Theme 3: The Regenerative Dimension - Red and Near-Infrared Light and Fibroblast Proliferation

The aim of this theme is to investigate the role of red and near-infrared light on the regeneration process, as well as fibroblast proliferation. This theme focuses on the effect of red and near-infrared light on the regeneration process and fibroblast proliferation.

The other extreme of the literature is the ability of light at lower doses and longer wavelengths to activate the host cells involved in wound closure. At the core of this process are the fibroblasts which secrete collagen and contract the wound matrix. Red and near-infrared (NIR) light has been consistently observed to increase the viability, growth and movement of fibroblasts under certain dosages. Hawkins and Abrahamse (2006) reported that irradiation of human skin fibroblasts with a helium-neon laser (632.8 nm) with a dose of about 5 J/cm² resulted in wound healing and enhanced proliferation and migration rates, while doses of 10-16 J/cm² led to a reduction of viability and an increase of DNA and membrane damage. Their previous work also separated low doses that stimulated from high doses that inhibited (Hawkins & Abrahamse, 2005).

This is a condition and wavelength dependent regenerative effect. Houreld and Abrahamse (2008) found that laser irradiation of diabetic-wounded fibroblasts led to a dose- and wavelength-dependent response, with short, visible wavelengths and lower doses favoring the process of closure and longer wavelengths and higher doses increasing apoptosis. More recent studies have revealed that different types of dermal fibroblasts respond differently to visible and near-infrared light, indicating that the regenerative response is not the same across cell types (Mignon et al., 2018). Mechanistically, the stimulation is associated with the same oxidative chemistry as mentioned above: Chen et al. (2011) showed that low-level laser therapy stimulated the redox-sensitive transcription factor NF- κ B in fibroblasts by ROS production, thus establishing a link between a controlled, sub-lethal oxidative burst and a proliferative signalling pathway. The second pole is provided by the regenerative theme: Light can promote the proliferation of fibroblasts, however within a very narrow range of low doses, and then only when applied in an oxidative dose that is a bit higher.

Some other characteristics of the regenerative literature should be stressed. First, the outcomes of the relevant wavelengths and doses are not limited to cell proliferation, but also encompass migration, collagen and growth factor expression, and accelerated in vitro wound closure—all of which are directly relevant to dermal repair—and are linked to the specific dose and wavelength. First, the outcomes of the relevant wavelengths and doses do not apply to simple cell proliferation, but to migration, collagen and growth factor expression, and accelerated in vitro wound closure, all of which are directly relevant to dermal repair and linked to the specific dose and wavelength, whereby Houreld and Abrahamse (2008) showed that the highest dose resulted in a suppression of basic fibroblast growth factor (bFGF) expression in diabetic-wounded cells. Secondly, the response is not only dependent on the total energy but also on the temporal distribution of the irradiation. As well, Hawkins and Abrahamse (2006) showed that repeated low-dose exposures may be beneficial for proliferation, while further high dose exposures would worsen the damage, suggesting that another parameter to optimize is the frequency of treatments. Third, cell context is important; fibroblast cells, which are the cells involved in chronic wounds, are not the same as healthy cells and therefore may reflect different behaviors than healthy cells, and parameters established in healthy models cannot be extrapolated to the pathological situation (Houreld & Abrahamse, 2008; Mignon et al., 2018). These are the nuances that signal the regenerative window is not one dimensional, but rather, spans a range of wavelengths, fluences, irradiance, fractionation and cell state.

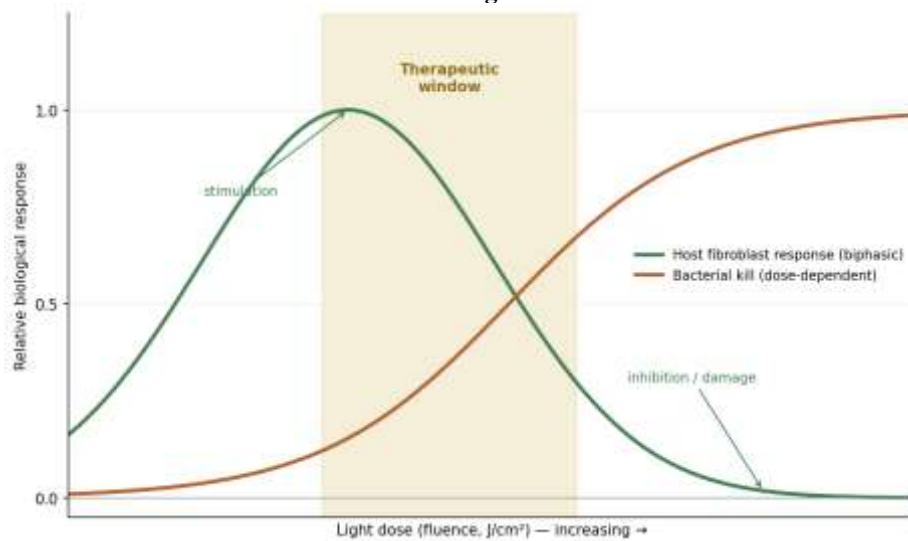
Theme 4: The biphasic dose-response and the therapeutic window

The two themes that have preceded this one are reconciled with what is perhaps the key organizing principle in the field, the biphasic dose response, which is commonly presented in the Arndt-Schulz format. Huang et al. (2009) compiled much of this evidence and found that as the dosage increases, the beneficial effect becomes less and less until a certain point, where the increase in dosage actually leads to a further decline in beneficial effect, thus creating an inverted-U-shaped curve. Excessive light causes excessive oxidative stress is cytotoxic, too much light below the threshold will not cause any response, and within the optimal range the response will be maximum. This law is the basis for the apparently paradoxical statements found in the literature about the same wavelength's healing and harmful effects, depending on the dose.

The dual therapeutic goal in this way sets out the optimisation problem. Bacterial eradication is on the high dose end of the axis, with long exposures to high levels of ROS being toxic, whereas fibroblast stimulation is on the low end of the dose axis, with short exposures to high levels of ROS acting as a signal. Hamblin (2017) also mentioned the additional complexity of PBM being able to increase ROS in normal cells, while decreasing ROS

in already oxidatively stressed cells, meaning the response of the host is dependent upon the redox state of the tissue. This relationship is shown graphically in figure 2, which illustrates the dose-dependent increase in killing of the bacteria and the biphasic response of the host cells; the region where the two curves are furthest apart is the zone of optimum killing.

Figure 2. Conceptual biphasic dose-response: host fibroblast stimulation versus dose-dependent bacterial killing.



Theme 5: Optimising Wavelength, Fluence, Irradiance, and Delivery

Combining the parameter evidence results in a picture in which wavelength and dose are playing complementary roles. The predominant chromophore engaged and the depth of light penetration into tissue depends primarily on the wavelength. The antimicrobial activity of blue light (400-470 nm) is quite effective due to the high absorbance of bacterial porphyrins, but may only reach the surface of the tissue and at high levels may have a greater potential for host-cell stress (Dai et al., 2012; Wang et al., 2016). Red and near-infrared light (around 630–980 nm) is able to penetrate deeper and more specifically activate the cytochrome-c-oxidase pathway of biostimulation, which is suitable for fibroblast proliferation (Avci et al., 2013; Karu, 1999). Fluence is the energy delivered per unit area, and then the exposure is placed on the biphasic curve; the irradiance, together with the exposure time, dictates the fluence, and both may have independent effects on the cell response.

Typical values reported in the experimental literature can be used to compare the two aims, as listed in Table 1. Low fluences (in the range of a few J/cm²) with red or near-infrared light are also effective for stimulation of fibroblasts, with approximately 5 J/cm² at 632.8 nm reported to be stimulatory and higher fluences reported to be inhibitory (Hawkins & Abrahamse, 2006; Houreld & Abrahamse, 2008). However, to achieve multi-log killing within biofilms to eradicate bacteria, blue light at much higher fluences (hundreds of J/cm²) is needed (Wang et al., 2016). The large dose difference between these doses is the key to the optimisation challenge and at the same time the opportunity: The regenerative window is far below the antimicrobial dose, so it makes sense to combine wavelengths, with high-dose blue light for antimicrobial activity, and low-dose red or near-infrared light for repair. This sequential or combined protocols, tailored to the bioburden and depth of the wound, naturally follow from the synthesised data, but there are both warnings in the literature against the use of multiple wavelengths in combination, which makes the dosimetry and interpretation more difficult (Hamblin, 2018).

The synthesis also suggests that the exposure is not fully described by the fluence. The dose of the radiation (the energy received/unit area) and the time of exposure to the radiation (the exposure time) can also affect the cellular response independently of the dose product. In an early demonstration of what is referred to as 'reciprocity failure' (equal total dose at different power densities produces different effects), Van Breugel and Bär (1992) showed that modulation of human fibroblasts by helium-neon produced the greatest effect when the power density and exposure time were greater. This poses two implications for optimisation. For regeneration, it means that a low irradiance for a prolonged period may be a better option than a high irradiance for a short period, as the latter may cause the cells to exceed the optimum stimulatory level. It means the bacteria are killed only by a cumulative dose that is large enough if they are to be eradicated, and this dose cannot be administered at a high enough irradiance to cause thermal injury. However, there are other variables such as mode of delivery (continuous or pulsed) and treatment interval that are inconsistently reported in the literature and additional factors that may be important, but for which it is difficult to draw firm conclusions.

The parameter which is most directly related to the selection of wavelength to the wound geometry is penetration depth. In tissue, light is scattered and absorbed, and for longer wavelengths, light is absorbed deeper, making red and NIR light attractive when the target is deep in tissue, while blue light is absorbed superficially (Avci et al., 2013; Dai et al., 2012). For a shallow, heavily colonised wound bed, superficial deposition of blue light is appropriate for surface biofilm; for organisms that penetrate deeper tissue or in tunnelling wounds, blue light may not be penetrating enough or a longer wavelength with an exogenous photosensitiser may be necessary and/or

mechanical debridement to expose the bioburden. Other beam properties like the spot size and uniformity of the radiation on the wound surface also influence whether the desired fluence is present in the entire wound, and that irregular wound surfaces are difficult to dose uniformly. The spatial aspects of this make optimisation not just about choosing numbers on a dose-response curve, but also ensure that the 3-D delivery of light is optimised to fit the 3-D shape of the wound.

Theme 6: Translational Barriers is biofilms, penetration, and safety

The last theme is about the hurdles between promise and reality in the clinic. The biofilm is the most prominent of these. The vast majority of chronic wounds contain biofilm that forms a matrix surrounding the organisms within that layer, which prevents the penetration of antimicrobials and protects embedded organisms, so light treatments need to be able to kill bacteria, as well as to penetrate and affect the architecture of the biofilm (James et al., 2008; Malone et al., 2017). The use of antimicrobial blue light has the potential to inactivate organisms within established biofilms, and may disrupt the structure of the biofilm, a benefit that is reinforced by the need for higher doses of blue light to achieve this effect, but also poses a concern for potential host-tissue tolerance (Wang et al., 2016).

The second barrier is tissue penetration. The wavelengths that are most effective for bacteria penetrate least and may not penetrate deeper tissue to kill bacteria, while red and near-infrared wavelengths penetrate deeper but are less effective antimicrobials. This physical trade-off puts limits on the use of a single wavelength and again favors combined strategies. Safety and selectivity is the third factor. The intrinsic presence of porphyrins in bacteria, and the brief range of singlet oxygen, give some selectivity, and, although blue light is much less genotoxic than the ultraviolet radiation, it is possible that oxidative damage to host cells could happen at antimicrobial doses (Dai et al., 2012; Hamblin, 2017). Last, the literature continuously identifies that the parameters of irradiation are not always accurately and completely reported, and in the absence of standardised dosimetry, it would be impossible to compare or reproduce studies, and optimised windows could not be reliably defined (Anders et al., 2015). The translational theme then balances the optimism of the mechanistic themes with a pragmatic overview of the engineering, biology and methodological challenges still to be overcome.

Theme 5: Optimising Wavelength, Fluence, Irradiance, and Delivery

Synthesising the parameter evidence yields a picture in which wavelength and dose perform complementary roles. Wavelength chiefly determines which chromophore is engaged and how deeply light penetrates tissue. Blue light (around 400–470 nm) is strongly absorbed by bacterial porphyrins and is therefore well suited to antimicrobial action, but it penetrates tissue only superficially and, at high doses, carries greater potential for host-cell stress (Dai et al., 2012; Wang et al., 2016). Red and near-infrared light (approximately 630–980 nm) penetrates more deeply and preferentially drives the cytochrome-c-oxidase pathway associated with biostimulation, making it appropriate for fibroblast proliferation (Avci et al., 2013; Karu, 1999). Fluence, the energy delivered per unit area, then positions the exposure along the biphasic curve, while irradiance and exposure time jointly determine fluence and may exert independent effects on the cellular response.

Representative values reported in the experimental literature illustrate the contrast between the two objectives, as summarised in Table 1. For fibroblast stimulation, low fluences in the region of a few joules per square centimetre with red or near-infrared light recur as effective, with roughly 5 J/cm² at 632.8 nm cited as stimulatory and higher doses as inhibitory (Hawkins & Abrahamse, 2006; Houreld & Abrahamse, 2008). For bacterial eradication, blue light at much higher fluences, on the order of hundreds of joules per square centimetre, is required to achieve multi-log killing within biofilms (Wang et al., 2016). The large separation between these dose regimes is the crux of the optimisation challenge and also its opportunity: because the regenerative window lies well below the antimicrobial dose, a strategy that combines wavelengths, using higher-dose blue light to disinfect and lower-dose red or near-infrared light to stimulate repair, is mechanistically coherent. Such sequential or combined protocols, calibrated to the wound's bioburden and depth, follow logically from the synthesised evidence, although the literature also cautions that combining wavelengths complicates dosimetry and interpretation (Hamblin, 2018).

The synthesis also indicates that fluence alone does not fully capture the exposure. Irradiance, the power delivered per unit area, and the duration over which it is applied can influence the cellular response independently of their product. Van Breugel and Bär (1992) reported that the power density and exposure time of helium-neon irradiation were more important than the total energy dose in modulating human fibroblasts, an early demonstration of what is sometimes called reciprocity failure, in which equal total doses delivered at different rates produce unequal effects. This has two consequences for optimisation. For regeneration, it implies that a low irradiance delivered over a longer time may be preferable to a brief, intense exposure of the same nominal fluence, because the latter risks pushing cells past the stimulatory threshold. For eradication, it implies that the high cumulative doses needed to kill bacteria must be delivered without generating thermal injury, which constrains the permissible irradiance. The mode of delivery, continuous versus pulsed, and the treatment interval are further variables that the literature treats as potentially significant but inconsistently reported, which limits firm conclusions.

Penetration depth is the parameter that most directly couples wavelength choice to wound geometry. Tissue is an optically turbid medium that scatters and absorbs light, and within the therapeutic range longer wavelengths generally reach deeper before being attenuated, which is why red and near-infrared light is favoured when the target lies below the surface, whereas blue light deposits its energy superficially (Avci et al., 2013; Dai et al., 2012). For a shallow, heavily colonised wound bed, the superficial deposition of blue light is well matched to

surface biofilm; for organisms harboured in deeper tissue or in tunnelling wounds, blue light may simply fail to reach them, and either a longer antimicrobial wavelength with an exogenous photosensitiser or mechanical debridement to expose the bioburden becomes necessary. Beam parameters such as spot size and uniformity of irradiation further affect whether the intended fluence is actually delivered across the whole wound, and irregular wound surfaces make uniform dosing difficult. These spatial considerations mean that optimisation is not only a matter of selecting numbers on a dose-response curve but of matching the physical delivery of light to the three-dimensional reality of the wound.

Table 1. Representative Light Parameters Reported for the Two Therapeutic Objectives

Objective	Wavelength domain	Fluence (illustrative)	Representative source
Fibroblast stimulation	Red (632.8 nm)	~5 J/cm ² (stimulatory; ≥10–16 inhibitory)	Hawkins & Abrahamse (2006)
Fibroblast stimulation	Visible–NIR (multiple)	Low doses; effect wavelength-dependent	Hourelid & Abrahamse (2008)
Bacterial eradication	Blue (415 nm)	Hundreds of J/cm ² (biofilm)	Wang et al. (2016)
Bacterial eradication	Blue (400–470 nm)	Dose-dependent multi-log killing	Dai et al. (2012)

Note. Values are illustrative figures drawn from the cited primary studies to convey the contrast in dose regimes; they are not pooled estimates and should not be read as clinical dosing recommendations. Reported parameters vary widely with organism, cell type, and exposure conditions.

Theme 6: Translational Barriers is biofilms, penetration, and safety

The last theme is about the hurdles between promise and reality in the clinic. The biofilm is the most prominent of these. The vast majority of chronic wounds contain biofilm that forms a matrix surrounding the organisms within that layer, which prevents the penetration of antimicrobials and protects embedded organisms, so light treatments need to be able to kill bacteria, as well as to penetrate and affect the architecture of the biofilm (James et al., 2008; Malone et al., 2017). The use of antimicrobial blue light has the potential to inactivate organisms within established biofilms, and may disrupt the structure of the biofilm, a benefit that is reinforced by the need for higher doses of blue light to achieve this effect, but also poses a concern for potential host-tissue tolerance (Wang et al., 2016).

The second barrier is tissue penetration. The wavelengths that are most effective for bacteria penetrate least and may not penetrate deeper tissue to kill bacteria, while red and near-infrared wavelengths penetrate deeper but are less effective antimicrobials. This physical trade-off puts limits on the use of a single wavelength and again favors combined strategies. Safety and selectivity is the third factor. The intrinsic presence of porphyrins in bacteria, and the brief range of singlet oxygen, give some selectivity, and, although blue light is much less genotoxic than the ultraviolet radiation, it is possible that oxidative damage to host cells could happen at antimicrobial doses (Dai et al., 2012; Hamblin, 2017). Last, the literature continuously identifies that the parameters of irradiation are not always accurately and completely reported, and in the absence of standardised dosimetry, it would be impossible to compare or reproduce studies, and optimised windows could not be reliably defined (Anders et al., 2015). The translational theme then balances the optimism of the mechanistic themes with a pragmatic overview of the engineering, biology and methodological challenges still to be overcome.

Another two aspects of translation are found repeatedly in the literature. The first is that oxidative photochemistry requires molecular oxygen. The Type II pathway and to some extent the Type I pathway, depend on oxygen as a reactant, while chronic wounds are often hypoxic, with the lack of oxygen that is important for non-healing of wounds also limiting the antimicrobial effects of light; this also favours local oxygenation as well as oxygen-independent damage. The second is the difference between laboratory promise and clinical proof. The majority of the best evidence comes from in vitro cell cultures and small-animal models; rigorous and well-powered clinical studies in human chronic wounds are relatively few, and reports from clinical trials in humans, such as with venous leg ulcers, have been mixed, partly due to the above-mentioned parameter heterogeneity (Avci et al., 2013). This will need to be achieved not only by improving the trials, but by standardising devices and reporting conventions, to get an effective parameter set once found and be able to repeat it across centres (Anders et al., 2015).

Discussion

Together, the six themes tell the story of a field in which the same rapid paradox reigns: oxidative stress can be a weapon against wound pathogens, and a messenger to recruit host repair. The apparent contradiction is resolved by the synthesis which makes the dose and the wavelength the governing variables. In small and short-lived amounts, ROS triggers redox-sensitive signalling (e.g. NF-κB) to stimulate the proliferation of fibroblasts and synthesis of the matrix (Huang et al., 2009; Chen et al., 2011), while in larger and more prolonged amounts, oxidatively breaks down bacterial cell membranes and nucleic acids. Optimisation is not a quest for a single magic

number, but a gradient management; stimulate cells in the host without exceeding their threshold for oxidative stress while maintain pathogen levels above their threshold for oxidative stress.

The most consistent approach suggested by literature from this point of view is divided approach. The shorter wavelengths are the most effective at the high doses of fluence, while the longer wavelengths are more effective at the low doses of fluence, so there is no single exposure that is optimal for both antimicrobial and regeneration purposes. A sequential protocol that uses higher doses of blue light to kill bioburden and disrupt biofilm is then followed by low doses of red or near-infrared photobiomodulation to stimulate fibroblast closure is thus the most natural approach, and is supported by the mechanism and dose-response data. This interpretation is in line with the general idea that the effect of PBM can be different, depending on the redox state of the tissue and on the type of cells (Hamblin, 2017; Mignon et al., 2018). It also changes the clinical target from maximising kill to maximising stimulation, to broaden and widen the therapeutic window between them.

The synthesis also helps to explain the conflicting results found in the field. The apparent inconsistencies in antimicrobial efficacy can be accounted for by the species-specific differences in endogenous chromophore content and antioxidant defenses (Dai et al., 2012; Wu et al., 2018), while the apparent inconsistencies that a particular wavelength can be both beneficial and detrimental are explained by the biphasic principle. Many of the differences in the literature are not actually contradictory but only differences in dose, wavelength, organism and cell model that have been hidden by the lack of complete reporting of parameters. This observation puts dosimetric standardisation to the forefront of science as a central issue, and, without consistent reporting of wavelength, fluence, irradiance, spot size, and exposure, the optimal windows for eradication and proliferation can only be mapped with some level of uncertainty (Anders et al., 2015).

The following practical and research implications can be drawn. Rigorous fully reported dose response experiments, in the same model and parameter space, are a priority for investigators who wish to evaluate antimicrobial and fibroblast outcomes both in biofilm and in vivo (reflecting the chronic wound) systems. The analysis is used for device development, and incorporates the use of multi-wavelength platforms that provide both an antimicrobial phase and a regenerative phase, with control of dosimetry for each phase. Protocols will have to be optimized for the type of wound, the degree of bioburden, the maturity of the biofilm, and the depth of tissue in order for these to be clinically translated, and not used as a 'recipe'. The ability of oxidative killing to resist resistance, which continues to be the strongest argument in the field, is justly used today as an additional advantage: In an era of antimicrobial resistance, a killing mechanism that bacteria are hard to escape is strategically useful, if the host tissue costs associated with its use can be managed (Dai et al., 2012).

One can attempt to imagine what the integrated protocol suggested by this synthesis might be like, but one should think of it tentatively, as a hypothesis to be tested, rather than a clinical recommendation. A first step antimicrobial phase would involve blue light (either alone or combined with an exogenous photosensitiser in deeper or less porphyrin-rich infections) based on the high fluences that have been demonstrated to be effective in inactivating biofilm organisms, while limiting the irradiance to avoid thermal damage and adjusting the dose based on the bioburden of the wound and the maturity of the biofilm. After the bioburden is reduced, a regenerative phase would involve applying red or near-infrared photobiomodulation (PBM) at low fluences, under the biostimulatory window, but repeated in a fractionated schedule to keep fibroblasts in the growth phase (not in the inhibitory window). The two phases are treated as two points on the same oxidative continuum, but in principle they are separable, and they can be individually optimised, with the practical consequence of this that the payoff of the two phases is a function of their two sets of wavelengths and dose. Importantly, this proposal has not yet been tested in combination, the evidence is only for each component individually, and the goal of the synthesis is to make the combined logic explicit and falsifiable.

There are two warnings for this positive interpretation. The first is about selectivity. The idea that light does not harm host cells is partially based on the fact that bacteria have more porphyrin than host cells, and the singlet oxygen has a short range, but the doses of antimicrobial light are high and during the antimicrobial phase, the same wounded, stressed fibroblasts that the regeneration phase wants to help may be especially vulnerable. Therefore selectivity is relative and dose dependent, and protocols need to be developed to ensure that the exposure of the host is kept within acceptable limits. The second is related to the evidentiary base. Much of the mechanistic understanding is based on monocultures and simple models, so the behavior of light in a complex, polymicrobial, hypoxic human wound with its complex host responses is not completely predictable from these data.

CONCLUSION

The objective of this qualitative synthesis was to gain insight into the mechanisms by which the application of PBM and other light-based therapies induce oxidative stress in CWP and how the parameters of the laser light could be optimized to kill bacteria and yet promote proliferation of the fibroblasts. The integrated evidence strongly suggests that both outcomes involve a single oxidative pathway; that the pathway is triggered by light, and that the two levers that direct the pathway are the wavelength (short-wavelength light for a lethal ROS pathway, and red and NIR light for a sub-lethal ROS pathway) and the dose (high fluence for a lethal ROS pathway, and low fluence for a sub-lethal ROS pathway). There is no single set of parameters that is optimal for both ends, but rather a combination and/or sequential wavelength-divided approach supplemented by more penetrating regenerative wavelengths and resistance resilient antimicrobial wavelengths optimized to the wound. The potential for realisation lies less in finding new mechanisms and more in disciplined and standardised dosimetry and in integrated experimentation where killing and healing are measured. Biologically coherent and

resistance-sparing, light induced oxidative stress is a new concept that offers a viable solution to the challenges of disinfection and regeneration of the chronic wound when the dose and wavelength are properly partitioned.

Limitations of the Review

This review is a qualitative evidence synthesis, which means that it does not produce primary data, but rather interprets and integrates prior studies, and thus conclusions reached are limited by the quality and comparability of the prior studies. Irradiation parameters were generally reported in a non-unified way and were often incomplete, which hindered the determination of optimal parameters with precision, and illustrative rather than pooled values were provided for the parameters, to avoid the false impression of a high degree of precision. No attempt was made to systematically search for seminal papers and papers that have been cited in the literature, and so there is a risk of selection and interpretive bias. The evidence cited stems largely from *in vitro* and animal models and may not translate to human chronic wound. It would be desirable when the primary literature adopts a consistent parameter reporting to be able to do a formal systematic review with a standardised dosimetric extraction for future syntheses.

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