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Biophoton Detection and Low-Intensity Light Therapy: A Potential Clinical Partnership

Joseph Tafur, M.D.,[1] Eduard P.A. Van Wijk, Ph.D.,[2,3] Roeland Van Wijk, Ph.D.,[2,4] and Paul J. Mills, Ph.D.[1]

Abstract

Low-intensity light therapy (LILT) is showing promise in the treatment of a wide variety of medical conditions. Concurrently, our knowledge of LILT mechanisms continues to expand. We are now aware of LILT's potential to induce cellular effects through, for example, accelerated ATP production and the mitigation of oxidative stress. In clinical use, however, it is often difficult to predict patient response to LILT. It appears that cellular reduction/oxidation (redox) state may play a central role in determining sensitivity to LILT and may help explain variability in patient responsiveness. In LILT, conditions associated with elevated reactive oxygen species (ROS) production, e.g. diabetic hyperglycemia, demonstrate increased sensitivity to LILT. Consequently, assessment of tissue redox conditions *in vivo* may prove helpful in identifying responsive tissues. A noninvasive redox measure may be useful in advancing investigation in LILT and may one day be helpful in better identifying responsive patients. The detection of biophotons, the production of which is associated with cellular redox state and the generation of ROS, represents just such an opportunity. In this review, we will present the case for pursuing further investigation into the potential clinical partnership between biophoton detection and LILT.

Introduction

Low-intensity light therapy (LILT) is showing promise in the treatment of a wide variety of conditions, including the treatment of delayed wound healing, arthritic pain, and acute stroke.[1–4] Our understanding of LILT mechanisms continues to progress, and we now know that LILT has the potential to accelerate ATP production and mitigate oxidative stress, which derives from excessive production of reactive oxygen species (ROS) or a lack of antioxidant activity.[5–7] Through red- and near infrared (NIR)-induced mitochondrial stimulation, these mechanisms participate in downstream immunomodulation in cells and tissue.[8–11] Coupled with reports of clinical safety and efficacy, this increased understanding continues to generate enthusiasm for LILT. As the field progresses, however, we must recognize that currently, in some individuals, it is difficult to predict clinical response. This is further complicated by the diversity of protocols and methodologies that have been utilized and that have reported mixed results in the treatment of a variety of conditions, e.g. osteoarthritis, myofascial pain, and carpal tunnel syndrome.[3,12–15] Nonetheless, consistent investigation has demonstrated that red and NIR low-intensity phototherapy can influence cells and tissues in a wavelength-specific, intensity-specific, energy dose-specific, and pulse frequency-specific manner.[10,11,16–19] These effects are generally oxygen dependent and involve the generation of ROS.[8,20,21]

As we clarify our understanding of relevant molecular mechanisms, it appears that cellular reduction/oxidation (redox) state may play a central role in determining sensitivity to LILT and may help to explain variability in patient responsiveness.[22–24] Conditions associated with pro-oxidant states, i.e. states associated with elevated ROS production, demonstrate increased sensitivity to LILT.[25] Cellular sensitivity to red and infrared light, probably at the level of cytochrome C oxidase, is influenced by cellular redox state.[22,23,26] Cellular growth phase, which may also correspond to cellular redox state, appears be another determinant of this sensitivity. *In vitro* and *in vivo*, the effectiveness of LILT varies with cellular growth phase.[2,6] Proliferating cells are in many cases more sensitive. HeLa cells, fibroblasts, and epithelial cells all demonstrate sensitivity to LILT, which is more pronounced during the proliferative cellular growth phase, and in each case, this proliferative phase is associated with elevated ROS production.[24,27–30] *In vivo*, such effects are further affected by the pathophysiologic state of the treated tissue, which may

[1]Department of Psychiatry, Behavioral Medicine Laboratory, University of California at San Diego, San Diego, CA.
[2]International Institute of Biophysics, Neuss, Germany.
[3]Leiden/Amsterdam Center for Drug Research, Analytical Biosciences, Leiden University, The Netherlands.
[4]Utrecht University, Utrecht, The Netherlands.

also correspond with cellular redox state.[31,32] Diabetic wounds, which are characterized by delayed wound healing, are an interesting case. For example, proliferating cells at the diabetic wound site are more responsive to LILT than non-proliferating cells.[33,34] Furthermore, compared to normally healing wounds, diabetic wounds are more responsive to LILT.[25] This difference is significant. In their study of LILT effects on burn healing, Al-Watban and Andres reported that light-emitting diode (LED) therapy at doses of 5, 10, 20, and 30 J/cm^2, respectively, influenced healing by 6.85%, 4.93%, 24.18%, and 25.42% in non-diabetic rats and 73.87%, 76.77%, 60.92%, and 48.77% in diabetic rats, relative to their controls, respectively.[34] This increased responsiveness may be the result of elevated ROS production known to be associated with diabetic hyperglycemia, although this proposed mechanism has not yet been confirmed.[35,36]

Curiously, LILT has been associated with further transient increases in cellular ROS production.[20] As aforementioned, we theorize that conditions characterized by pathophysiological oxidative stress may demonstrate increased cellular sensitivity to LILT. The use of LILT in the treatment of cardiac and cerebral ischemia, both characterized by oxidative stress, provides some insight into the beneficial effects of LILT in settings of elevated ROS production.[4,37,38] In such conditions, the use of LILT might appear counterproductive, as it is associated with further increases in ROS production. However, animal studies investigating the use of LILT in the treatment of experimentally induced cardiac and cerebral ischemia indicate otherwise.[4,37,38] In this research, LILT results in subsequent improvements in tissue survival and function as compared to control conditions. LILT-induced improvements in these conditions have been attributed by Oron et al. to rapid elevation of ATP content, increased angiogenesis, and furthermore, to increased anti-apoptotic activity, heat shock proteins, and total antioxidants.[5] These improvements draw a comparison with more well-established protective cellular mechanisms, which have also been studied in the setting of cardiac and cerebral ischemia, namely, those involved in the highly conserved cellular stress response induced by heat shock, pre- and post-ischemic conditioning, and oxidative stress.[39–46] This stress response is characterized by increases in anti-apoptotic activity, heat shock proteins, and total antioxidants.[46] In the treatment of cardiac ischemia, LILT might best be compared to the experimental technique of post-ischemic conditioning, in which it has been shown that ischemic myocardial injury can be reduced by cycles of re-occlusion during myocardial reperfusion.[46] In both cases, the subsequent mitigation of oxidative stress is in part initiated by the further generation of ROS. Such increases in ROS production likely participate in intracellular signaling by acting on a number of redox-sensitive proteins, including redox-sensitive transcription factors.[24,46] Prior investigations show that LILT can alter the expression of a variety of genes, including genes known to directly or indirectly play roles in the enhancement of antioxidation.[28]

As cellular conditions involving elevated ROS production appear to be associated with increased LILT sensitivity, assessment of tissue redox conditions *in vivo* may prove helpful in identifying responsive tissues. A noninvasive redox measure may be useful in advancing investigation in LILT and may one day be helpful in identifying responsive patients. The detection of biophotons, whose production is also associated with the production of ROS and the cellular redox state, represents just such an opportunity. Biophoton detection and analysis are currently being utilized to noninvasively measure tissue redox conditions *in vivo*.[47–49] In this review and position paper, we will introduce relevant biophoton research focusing on the relationship between biophoton emission and conditions of oxidative stress. We also include some discussion of the initial context of biophoton investigation. Moreover, we will present the case for further investigation into the potential clinical partnership between biophoton detection and LILT.

Biophoton Detection

Over the last few decades, advances in photodetection have confirmed that many, if not all, living systems emit very low levels of visible and near visible (ultraviolet [UV] and near infrared [NIR]) photons.[50,51] These photobiological emissions have been described as spontaneous ultraweak chemiluminescence, spontaneous photon emission (SPE), and, more succinctly, as biophoton emission.

Visible and NIR spectrum biophotons, linked to electron-excited states associated with the generation of ROS, are most relevant to LILT and to this discussion. In contrast, the origins of UV biophotons are less clear, and consequently, less clinically useful at this time. UV biophotons are theorized to be derived from DNA conformational changes, amplified radical recombination reactions, delayed branch chain reactions in amino acids, and/or direct emitters.[52,53] Future research might reveal a broader role for UV biophotons in cell biology.

Biophoton emission is biochemically distinct from the more well-known phenomena of bioluminescence. Bioluminescence, as in fireflies, is typically visible and involves specialized enzymatic mechanisms, i.e. luceferin–luciferase, to provide luminescence. In contrast, biophoton emission is much weaker and is generally reported to be less than 1000 photons per second per cm^2, several orders of magnitude below the accepted visible level.[54]

Biophoton emission is closely correlated with the production of ROS and the oxidative status of living systems. Recent advances in photodetection have made it possible to analyze biophoton emission. This methodology detects and analyzes biophotons produced by metabolic reactions in living cells. Since the early 1980s, scientists have recorded biophoton emission from mammalian liver, heart, lung, nerve, and muscle tissue.[55–62] This has been accomplished with photomultiplier tube technology, i.e. photomultipliers. These photomultipliers are extremely sensitive detectors of light in the UV, visible, and NIR ranges of the electromagnetic spectrum. These detectors multiply the signal produced by incident light by as much as 100 million times, enabling single photons to be detected individually when the incident flux of light is very low.[63] In collecting this data, it must be emphasized that the number of photons registered depends on a variety of instrumental characteristics, including the distance between the sample and the photomultiplier and, moreover, the spectral sensitivity of the photomultiplier.

Photomultipliers have been used to further analyze biophoton emission from total organ brain and liver homogenates in cell fractionation studies.[64] In these analyses,

mitochondria and submitochondrial particles have been shown to be predominant sources of cellular biophoton emission.[65–67] Furthermore, in fractionated hepatic cell homogenates, microsomes (vesicular fragments of the endoplasmic reticulum formed after the disruption and centrifugation of cells) have also been shown to be predominant sources of biophoton emission.[66,68–72] In hepatic cells, microsomes contain the cytochrome P450 enzymes, involved in oxidative metabolism. In mitochondria and microsomes, biophoton emission requires a membrane-bound electron transfer system and can be optimized in the presence of oxygen. In order to identify more precisely the molecular source of biophotons, spectral analysis has been applied extensively to biophoton emission in liver, brain, lung, and heart cells and hepatic microsomal fractions.[61,64,65,68,70] Various emission bands in the range of 400–700 nm have been found, and the data suggest that singlet molecular oxygen, formed during free-radical processes accompanying lipid peroxidation, may be a major source of biophoton emission. Interestingly, the ROS singlet oxygen is also thought to act as a signaling molecule in the cellular response to LILT photostimulation.[73] For a more comprehensive account of lipid peroxidation, including the oxidizing species triggering and participating in this process, the reader is referred to Boveris et al. and Halliwell and Gutteridge.[59,74]

As biophoton emission has been related to the utilization of oxygen, the generation of ROS, and the production of electronically excited states in biological systems, it was suggested that biophoton detection could be used as a tool for the investigation of radical reactions and oxidative stress. Many biochemical techniques, which focus primarily on the measurement of biological lipid peroxidation, are now available to assess oxidative stress in living systems.[75] However, many of these techniques are invasive and inappropriate for clinical application. In contrast, biophoton emission provides, on a non-invasive basis, a signal of oxidative metabolism and ROS steady-state concentration that is readily and continuously detectable. It is possible to continuously monitor the metabolism of organs *in vivo* with biophoton detection techniques. Thus, in the assessment of *in vivo* redox status, biophoton detection may become more useful than other assays, including indirect assays of lipid peroxidation, such as glutathione release, electron-spin-resonance techniques, or assays assessing the evolution of hydrocarbons or malondialdehyde accumulation.[76–83]

Furthermore, as biophoton detection can be developed for imaging and spatiotemporal analysis, it offers additional perspectives. Two-dimensional biophoton imaging of a rat brain *in vivo* was achieved in 1999.[84] This was done using a highly sensitive, ultra-low-noise charged-coupled device (CCD) camera system. Kobayashi et al. demonstrated that spatiotemporal biophoton emission in the rat brain is correlated with cerebral energy metabolism and oxidative stress.[84] Biophoton emission intensity in the exposed rat brain was associated with cerebral blood flow and ROS production *in vivo*. Furthermore, work with subsequently sliced rat brains suggested that observed biophoton emission originated from the energy metabolism of the inner mitochondrial respiratory chain through the production of ROS. At that time, authors remarked that the imaging of biophoton emission from a brain constituted a novel method with the potential to extract pathophysiological information associated with neural metabolism and oxidative dysfunction. Kobayashi et al. credit Boveris and Cadenas et al. with suggesting the potential usefulness of biophoton detection for noninvasive monitoring of oxidative metabolism and oxidative damage in living tissue.[85,86]

Biophoton detection techniques have also been used to record ultraweak photon emission from superficially transplanted tumors.[87–89] Recently, a CCD camera system was used to record two-dimensional biophoton images from tumors transplanted in mice.[90] In these images, recorded biophoton emission distinguishes transplanted tumors from surrounding tissue. This distinction may be due to increased ROS production (including singlet oxygen, superoxide radicals, hydrogen peroxide, and hydroxyl radicals) associated with carcinogenesis and tumor promotion.[91] At this point, this recording technology is unable to demonstrate discrete tumor boundaries as biophoton emission spreads from areas of higher emission in this two-dimensional data. Additional studies will be required to further establish the accuracy of localizing biophoton tissue sources. This tumor research also initiates needed discussion addressing biophoton tissue penetration and the depth from which biophotons can be detected. More research needs to be done in this area in order to assess the transparency of living tissue with regard to ultraweak biophoton intensities.

Biophotons and LILT

Visible and NIR biophotons and red and NIR LILT share some interesting parallels. Both involve visible and NIR photons, the mitochondrial electron transport chain, and the generation of ROS, in particular, singlet oxygen. As previously reviewed, LILT can stimulate cellular metabolism, presumably through photostimulation of elements of the mitochondrial electron transport chain.[73] Furthermore, LILT photostimulation is thought to result in electron-excited states and the increased generation of ROS.[20]

Mitochondrial ROS generation is likely one of the primary mechanisms involved in LILT. As mentioned, Pal et al. demonstrated that, in human fibroblasts, low-intensity helium–neon laser irradiation-induced proliferation was associated with real time transient increases in ROS production.[20] Antioxidant mechanisms have been shown to inhibit such LILT effects, further implicating the role of ROS in LILT signal transduction.[23]

The mitochondrial electron transport chain is a major source of visible and infrared biophotons. In fact, the metabolic processes involved in producing electronically excited states in biophoton-producing molecules are generally derived from oxidative metabolism accompanied by the production of ROS.[48,92] Boveris et al. report that "hydroperoxide-supplemented cytochrome c provides a chemiluminescent model system suitable for the elucidation of some of the molecular mechanisms responsible for light emission".[85] Biophoton emission increases with increased generation of ROS by the mitochondrial electron transport chain.

Some researchers report that LILT and biophotons share another parallel. Like LILT, biophotons may influence cells through some form of photobiostimulation.[50,93] At first glance, this is an appealing idea yet very difficult to conceptualize, as the two phenomena differ vastly in their functional intensities. Low-intensity lasers and LEDs are effective

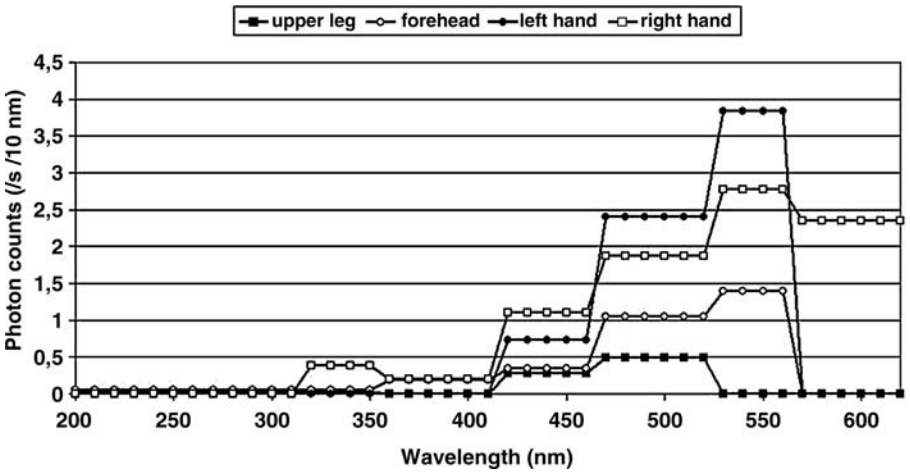

FIG. 1. Human biophoton emission measured in a single subject. Spectral distribution of spontaneous emission of different body sites of subject A: right anterior upper leg, forehead, left hand, and right hand (Reprinted with permission from *Forschende Komplementarmedizin und klassische Naturheilkunde = Research in Complementary and Natural Classical Medicine*, copyright held by S. Karger GmbH, Freiburg).[123]

at an intensity window whose minimum is several orders of magnitude greater than the intensity of recorded biophoton emission. In our current understanding, this makes it difficult to link biophoton-induced effects to LILT mechanisms. Nonetheless, this concept is derived from early biophoton investigations, which explored the possibility of endogenous intercellular photocommunication. This concept was born in the 1920s when A.G. Gurwitsch first described what he termed as "mitogenic radiation".[94] Gurwitsch reported experimental evidence that demonstrated that dividing yeast cells (*Saccharomyces cerevisiae*) emit UV light and can induce mitosis in a chemically separated population of *S. cerevisiae* cells. This was the first indication that an optical (physical) interaction might occur between cells. For decades, a number of researchers attempted to replicate this work and failed.

Despite the lack of convincing evidence, several laboratories in different countries continued to pursue systematic investigation into this matter, using both biological techniques and, as technology permitted, physical methods of photodetection.[95–107] As his controversial research inspired this more thorough investigation, Gurwitsch's initial work is regarded by many as the foundation for research into the role of biophotons in intercellular communication.

Subsequent technological advances in photodetection have now been applied in studies of biophoton-mediated intercellular communication. In 1991, Grasso et al. revived "mitogenic radiation" by demonstrating that *S. cerevisiae* biophoton emission appears to be capable of inducing higher rates of gemmae formation during *S. cerevisiae* self-irradiation experiments.[108,109] Research into optically mediated intercellular communication has been conducted primarily with microbes and has been reviewed extensively by Trushin.[93,110]

Others have explored the potential role of biophotons in intercellular interactions in neutrophils, which are incidentally also sensitive to LILT.[94,111] Low-intensity lasers can be used to induce the well-described oxidative burst in neutrophils, which occurs *in vivo* during phagocytosis.[112] During neutrophil phagocytosis and oxidative burst, the generation of ROS, involving excited singlet oxygen and excited carbonyl groups, leads to biophoton emission.[112,113] This emission has been investigated for its potential role in optically mediated neutrophil interactions.[111] Although beyond the scope of this discussion, some biophysicists theorize that such communication might involve complex biophysical mechanisms beyond those currently established in photobiology.[50,114] At this point, the theory that biophotons, through some form of photobiostimulation, may influence chemical reactions and cellular behavior needs further verification.

Human Biophoton Detection

Over the last 10 years, human biophoton emission has also been detected and analyzed. Human biophoton emissions demonstrate spatiotemporal differences and are linked to ROS production and oxidative stress.[115–118] Cohen and Popp have conducted long-term systematic research of biophoton emission from the hands and forehead *in vivo* using a moveable, hanging photomultiplier.[47,119] This work includes investigations into the effects of disease states on biophoton emission.[115,120] This initial work provided the basis for a larger systematic study initiated in 2003. This research has yielded information on: (a) procedures for reliable measurements and spectral analysis; (b) the anatomic intensity of emission; (c) associations with biological rhythms; (d) physical and psychological influences on emission; and (e) emission in health and disease. Systematic, multisite recordings utilizing the moveable photomultiplier system with healthy subjects present evidence for a "common" human anatomic intensity emission pattern (Fig. 1).[121–123] It must be noted that the mean (±SEM) background noise was 6.0 ± 0.4 cps (photon count rate per second). This suggests that, under the given experimental conditions, the basic spontaneous emission of the body lies in the range of 15 cps (less than 1 cps/cm^2 skin surface). The reliability of assessing anatomical differences at this scale is closely related to the recording procedure. Thus, an adequate recording duration necessitates both the registration of as many anatomical locations as possible, within a reasonable time period, and high accuracy in order to reliably distinguish between the emission intensities of different locations. These two variables, however, act in opposite manner. Using statistical analyses, it has been concluded that recording times of 120 s, with emission counts grouped by seconds, give reliable values that can be used for these analyses. Differences greater than 1.1 cps between two locations are found to be statistically significant ($p < 0.01$).[123]

Despite this common anatomic pattern, absolute intensities of emission have been shown to vary widely between subjects, in some cases by up to a factor of 5. Analysis of

biophoton emission from the hands suggest that emitted photons are generated from both the skin surface and interior sources. Spectral analysis documents major spontaneous emission at 470–570 nm, indicating a specific electron-excited state.[123] Excited carbonyl groups, perhaps generated by reactions between excited singlet oxygen molecules and unsaturated lipids, may be responsible for this emission. Furthermore, it has also been shown that biophoton emission is altered in the presence of antioxidants.[47] Other data has confirmed that human biophoton emission is oxygen-dependent and reduced in hypoxic conditions.[123]

A Potential Clinical Partnership

As most clearly described by Kobayashi et al., (visible and infrared) biophoton emission reflects the pathophysiological state with respect to ATP production and susceptibility to oxidative stress, which derives from excessive production of ROS or a lack of antioxidant activity. [48] Biophotons are thus a window into the redox state of the mitochondrial electron transport chain.

Biophoton emission from the mitochondria may similarly indicate localized redox conditions relevant to the mitochondrial electron transport chain and its light sensitive elements. Thus, biophoton emission seems uniquely suited to noninvasively characterize redox conditions related to low-intensity light sensitivity at the level of the mitochondrial electron transport chain.

The potential for clinically relevant *in vivo* human biophoton detection now exists. Although individual and anatomic variation need to be further clarified, skin-surface measurement of biophotons can be used to assess localized oxidative stress in superficial tissues. This information may be clinically useful in LILT. Biophoton emission might indicate mitochondrial redox conditions and thus potential sensitivity to LILT. Biophoton detection and analysis should therefore be investigated in connection with LILT clinical response. Investigating the relationship between biophoton emission, LILT, and wound healing (given the superficial source of biophotons) seems a reasonable starting point. In order to test the relationship between biophoton emission, redox status, LILT sensitivity, and clinical response, we suggest a study of LILT in wound healing, which would include interval assessment of biochemical redox markers and biophoton emission. Perhaps, biophoton measurement will be helpful in advancing LILT, and conversely, perhaps LILT will help us to further explore the now detectable world of biophotons.

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Author Disclosure Statement

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Address correspondence to:
Joseph Tafur, M.D.
University of California at San Diego
Stress Physiology Laboratory
9500 Gilman Drive
La Jolla, CA 92093-0804

E-mail: jtafur@ucsd.edu