Laser Photobiomodulation: Models and Mechanisms

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INTRODUCTION

The overwhelming clinical evidence of the clinical effectiveness of low-level laser therapy (LLLT) therapy has been balanced by few reports of equivocal or nonefficacious studies.[1-3] A major deterrent to the popularity of low-power lasers in various biomedical applications has been the lack of our understanding of precise mechanisms mediating the underlying biological responses. The aim of this article is to provide an overview of the various known biological mechanisms in LLLT and highlight the discovery of a recent mechanism describing LLLT-mediated activation of a latent growth factor complex, latent transforming growth factor-β1 (LTGF-β1) in stimulating oral wound healing.[4] This review is divided into four sections as follows: (1) Current understanding of photobiomodulation (PBM); (2) Critical parameters for photobiomodulation; (3) The nexus of inflammation and healing; and (4) Novel PBM mechanism involving LLLT activation of LTGF-β.

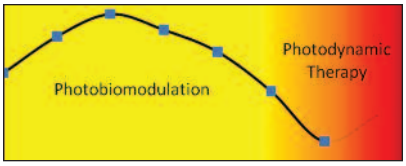

Figure 1: Laser dose that has a therapeutic regimen in the photobiomodulation segues into the destructive regimen termed photodynamic therapy.

CURRENT UNDERSTANDING OF PHOTOBIOMODULATION: MECHANISMS

As the pleiotropic effects of LLLT were better understood, they were collectively termed photobiomodulation to emphasize the role of light in the processes.[5] While there is a distinct low-level laser dose regimen within which the positive stimulatory effects of LLLT are beneficial, there also appears to be an ill-defined upper limit above which inhibitory-deleterious effects are clearly evident. The low-level laser dose encompasses the therapeutic PBM regimen and the higher doses constitute the photodynamic therapy (PDT) regimens (Figure 1). It is prudent to point out in these cases that PDT occurs with endogenous photosensitizers, unlike conventional antitumor therapy that utilizes exogenous photosensitizing dyes, and can produce significant, often irreversible biological damage.

Our current understanding of PBM mechanisms is largely focused on classical photophysical processes involving absorption of photonic energy and the subsequent photochemical process involving conversion of the absorbed radiant energy into highly reactive chemical intermediate species such as the Reactive Oxygen Species (ROS). The subsequent downstream photobiological responses have been extensively studied in the deleterious PDT regimen specifically when large amounts of ROS are generated.[6-7] Only recently the profound, wide-ranging, beneficial effects of these ROS mediators have been carefully elucidated.[8-10] Figure 2 summarizes our current understanding of the

ABSTRACT

The ability to modulate light-mediated biology has been termed *photobiomodulation* (PBM) and there are many reports on the potential clinical applications of this novel, noninvasive therapeutic modality. Despite these evidences, a major deterrent to its widespread application has been the absence of precise molecular mechanisms and thus the inability to apply standardized therapeutic treatment parameters to individual clinical scenarios. This review focuses on the current state of knowledge of known PBM mechanisms and proposes a conceptual model to assess biological effects. Further, a recently described mechanism involving low-power laser activation of a latent growth factor complex, TGF-β, in oral mucosal wound healing is presented. As our current understanding of the biological effects of low-level laser therapy (LLLT) is better understood, this modality can provide significant utility as a potent clinical tool.

KEYWORDS

Low-level laser therapy, low-level light therapy, photobiomodulation, TGF-β, biostimulation, photodynamic therapy, wound healing, reactive oxygen species (ROS)

molecular mechanisms of PBM, emphasizing the three distinct hierarchical phases that demonstrate the complex spatial and intricate nature of laser-biological interactions. The primary photon interaction involving various known and unknown photoacceptors can occur in any (likely multiple) levels that result in generation of extremely reactive and transient ROS which can diffuse rapidly and react with various components at various cellular levels inducing specific biological responses.[11] The extracellular milieu interactions involving activation of growth factor ligands or at the level of the cell membrane receptor activation could activate specific signal transduction pathways. The effects of lasers on mitochondria (specifically affecting the ROS and adenosine triphosphate (ATP)-mediated processes) have been extensively investigated. The transcription factors nrf-2, NFκB, cfos, and cJun are well-known antioxidant transcription factors mediating early transcriptional responses following cellular stress. This list is not meant to be all-inclusive; and, as we unravel newer interactions of photonic energy with various biological molecules, it is possible to envision the physical conformation or changed functional chemical state of biological molecules being directly modulated by photonic energy.

CRITICAL PARAMETERS FOR PHOTOBIOMODULATION: CONCEPTUAL MODELS

The four key variables in defining therapeutic dose are wavelength, total energy density or dose deposited-fluence (J/cm^2), power density of irradiation-irradiance (W/cm^2), and time. The effect of wavelengths in PBM is touted as the most well understood as it supposedly correlates precisely with the photophysical (absorption and scattering) and subsequent photochemical (ROS generation, change in chemical state such as phosphorylation) effects that can be rigorously measured. This is largely true for well-known photosensitive molecules such as endogenous flavins, porphyrins, cytochrome-*c* oxidase, among others.[5, 12] However, the PBM effects at nonabsorbing wavelengths, especially in the infrared range which are often preferred for their enhanced tissue penetration, remain unexplained. Thus, due to the complexity of the biological system, the therapeutic PBM parameters do not seem to correlate directly with simpler physical or chemical factors. Along with the wavelength, a common query is significance of the nature of the photonic source used. While traditionally broad light sources with filters were routinely used in early PBM experiments, the advent of cost-effective diode lasers and nonlaser light-emitting diodes (LEDs) have made these latter sources readily available. It should be pointed out that though the LEDs and filtered light sources clearly have discrete potent biological effects, for reasons yet unclear, lasers appear to have significantly improved efficacy at similar wavelengths and energy densities.

As there is not clear consensus on the precise PBM parameters, the following analogy for the three PBM parameters (fluence, irradiance, and time) is suggested. The aim here is to provide a conceptual framework for therapeutic PBM dose to aid laser clinicians and researchers unravel the critical variable for therapeutic applications. To begin with the simplest scenarios, we compare laser energy to fluid flowing from a tap and the biological system as an empty bucket on a balance where the biological system is at rest, in a nonequilibrium, steady state (Figure 3). The wavelength would be akin to the kind of liquid coming forth and may be compared to various fluids of

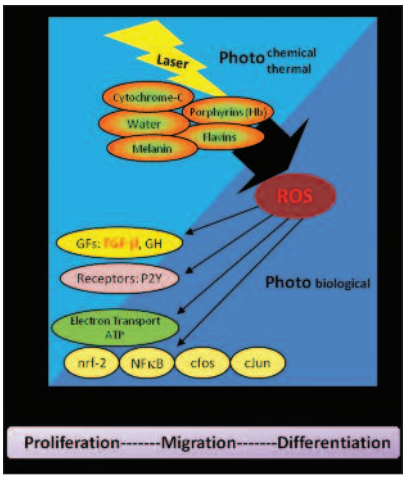

Figure 2: Current scheme of known PBM mechanisms demonstrating the putative cell-tissue compartments of photonic energy interactions. Abbreviations: GFs: Growth Factors; Hb: Hemoglobin; TGF-β: Transforming Growth Factor Beta; GH: Growth Hormone; P2Y: G-protein coupled purinergic receptor; ATP: Adenosine-5-triphosphate.

varying density like oil, milk, or water. For the sake of simplicity, we will assume the optimal wavelength based on the inherent photosensitizer in this system would be analogous to plain water.

The empty bucket needs to be 'filled'; in other words, there needs to be a minimal threshold energy to activate a system response. In this scenario, irrespective of the time variable of water coming out (flow mode and amount), there will be a clear biological response as long as the bucket is eventually filled optimally. This model can be termed a ***Balanced Bucket Model*** illustrating the critical role of fluence (J/cm^2) over the other two parameters of time (emission mode) and power density or irradiance (W/cm^2) (Figure 3A).

If the bucket is 'leaky' such that the rate of water loss equals the rate of inflow, then the critical parameter to elicit a biological response will depend on the amount of flow or irradiance but still needs to achieve a threshold activation energy state. This model

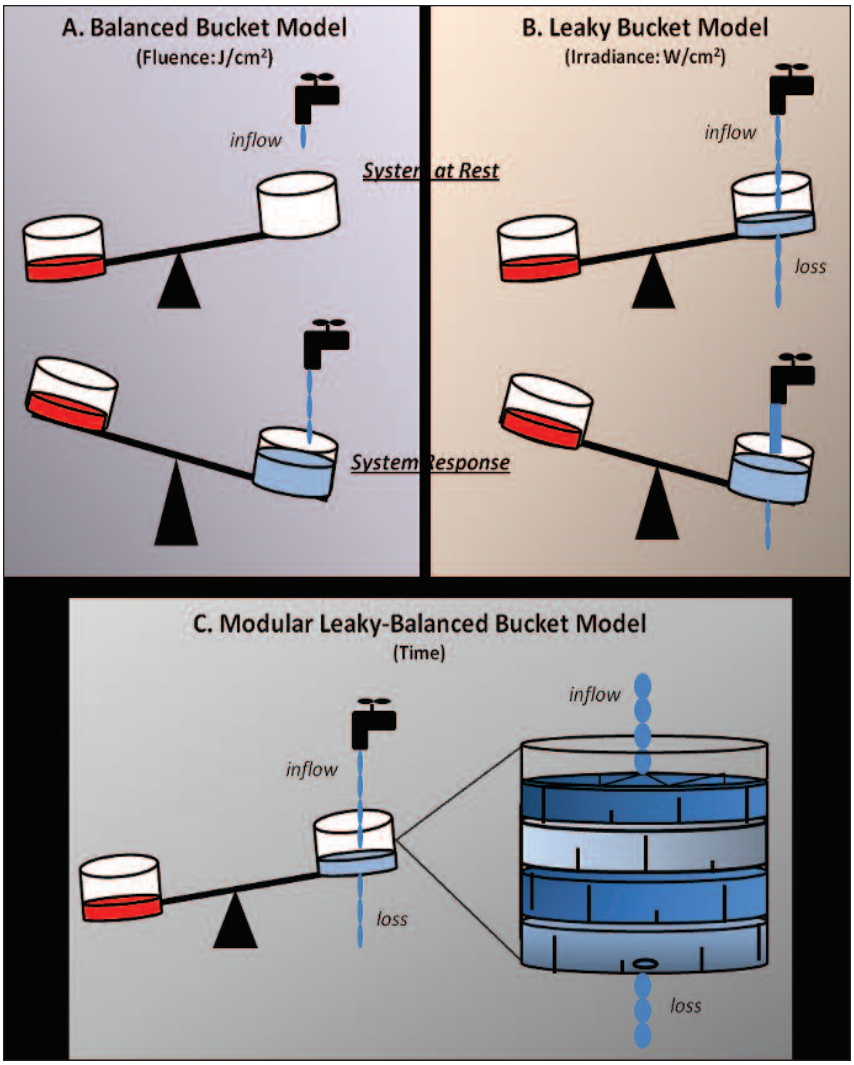

Figure 3: Proposed models to define role of the three key parameters for PBM, namely, fluence (graphic A), irradiance (graphic B), and time (graphic C.)

can be termed the ***Leaky-Balanced Bucket Model*** that emphasizes importance of irradiance as well as fluence (Figure 3B).

The third parameter is much more complicated to conceptualize. We could assume that the bucket, rather than being a single unit (as in the above 2 models), is a compartmentalized, modular system with connectors of varying sizes (differing inter-flow rates). We would term this the ***Modular Leaky-Balanced Bucket Model*** (Figure 3C). We conventionally refer to the time variable in PBM with respect to the tissue relaxation time but its precise physical, chemical, or biological nature is unclear. Rather than using this ill-defined tissue characteristic, it would help to ascribe the lag and recovery of responsiveness (system elasticity) to the various biological rate reaction constants (the inter-module connectors in this model) as well as dynamic concentrations within each module. The other two aspects where the significance of time can be key are the inflow rate and mode for irradiance (as discussed above) and the loss rate (leakiness, energy dissipation) which is inherent to the system and hence the terms 'leaky' and 'balanced' in this model. Both of these latter parameters can be potentially compensated for by adjusting the irradiance (inflow) or nature of energy deposition (continuous wave or pulsing frequency).

While these simplistic models are not meant to be all-encompassing, it is hoped that they would serve as a starting point in our current understanding of precise PBM parameters. Studies designed to test these three parameters independently would best unravel their individual roles and indicate the overall significance for each biological response in a given clinical scenario. It must be noted in the models that time as the fourth variable is already intertwined with irradiance; and that quantity could be extremely difficult to parse out experimentally unless precise multiple measurable biological end points are carefully defined *a priori*. It is also prudent to state that the current literature evaluating these parameters singularly have produced equivocal and sometimes beneficial results. However, often the results show either no or deleterious biological effects; the conclusion is that each biological process has distinctly defined end points, and will need optimization of these individual variables for therapeutic benefit.[3, 13-14]

THE NEXUS OF INFLAMMATION AND HEALING

Wound healing has been compared to both embryonic development and tumors.[15-19] This unique pathophysiological process has distinct phases that have characteristics of both embryonic development (exquisite control over biological processes) as well as malignant transformation (breakdown of control mechanisms). An ideal outcome of wound healing, besides structural reconstitution, is complete remodeling and functional restoration leading to regeneration that may (*epimorphosis*) or not

(*morphallaxis*) involve cell proliferation.

The complete absence or paucity of a well-developed inflammatory response is often cited for the regenerative capabilities of amphibians or scarless, regenerative fetal healing.[20-21] However, it is also well understood that routine wound healing must include inflammation. Following hemostasis, inflammation precedes the biological processes of angiogenesis, matrix synthesis, epithelial closure, and remodeling of connective tissue to restore form and function.[22] The inflammatory response not only has a central role in cleaning up the wound environment via phagocytosis of resident debris and foreign bodies including microorganisms, but also the inflammatory cells bring in potent chemical cues to promote the latter phases of healing, including cell recruitment, synthesis, and remodeling. It has been clearly shown that both the absence or presence of chronic and persistent inflammatory responses results in poor healing, thus emphasizing the key role of a transient, robust, and self-limiting role for inflammation in wound healing.[23-26] Further, the critical parameter defining the quality and quantity of inflammation is the extent and nature of the inciting damage.

NOVEL PBM MECHANISM INVOLVING LLLT ACTIVATION OF LTGF-β

Transforming Growth Factor-β (TGF-β) is a multifaceted growth factor that plays a key role in many biological processes from development to physiological homeostasis, and malignancies.[27-28] Despite having a broad functional range of influence, TGF-β effects on individual cell populations are context-dependent effects enabling exquisite specificity in modulating responses. Wounds are particularly abundant in TGF-β from both the initial platelet aggregate as well as infiltrating inflammatory cells, especially the monocytes/macrophages.[29]

TGF-β has been shown to be a potent chemo-attractant for the early inflammatory neutrophils as well as the subsequent inflammatory cells, specifically lymphocytes.[30] Although the overall effect of TGF-β on the secondary inflammatory milieu – specifically T lymphocytes – is largely inhibitory, it plays a prominent role in the resolution and remodeling of the wound tissue by promoting keratinocyte, endothelial, and fibroblast migration as well as matrix synthesis.[31] Interestingly, although there are few differences in various molecular and biochemical factors between skin and mucosal wounds, the ratio of TGF-β3 (among the three TGF-β isoforms) to TGF-β1 were found to be significantly increased in mucosa, suggesting a possible role for the less scarring evident in oral wounds.[32-33] Assessing the literature, we found striking similarities between the reported biological effects of low-power lasers and exogenous treatment with TGF-β. Among the prominent effects reported were increased synthesis of matrix constituents like fibronectin,[34-35] collagen,[36-37] myofibroblast transformation,[38-39] immunomodulation,[40-41] angiogenesis,[42-43] neurotrophic effects,[44-45] and a heat shock protein response involving Hsp70.[46-47] We also found two reports demonstrating increased TGF-β mRNA following low-power light[48] and low-power laser energy[49] in rats.

In a recent study, we first examined the PBM effects of an infrared laser in human subjects undergoing multiple extractions prior to rehabilitation with complete dentures.[4] The presence of multiple sites in the same patient facilitated randomization of control and laser-treated sites to either jaw, allowing each patient to act as his or her own control. This is critical in wound healing assessment as it is well known that regional and systemic factors can have a significant impact on healing outcomes in individual subjects even with very well-matched characteristics. The limitation of this model is that systemic factors, if perturbed by the treatment, can potentially affect the control sites. The study was performed with a 904-nm GaAs laser (Ora-laser1010, Oralia medical GmbH, Konstanz, Germany), a 10-mW unit with an aperture of 100 microns used in contact at 3 J/cm^2 for 5 min in oral extraction wounds. On the scheduled recall day, a small soft tissue biopsy was taken from the healing site and assessed by histology. The subjects were followed for a period of 2 years following complete oral rehabilitation and no untoward effects were observed. Routine and special stains were performed and various healing parameters such as inflammatory cell infiltrate, angiogenesis, matrix synthesis and reorganization were assessed. The results demonstrated a beneficial effect of LLLT on oral soft tissue healing.[4]

Having established a LLLT-accelerated oral healing model, we performed immunohiostochemistry on the healing tissue samples to evaluate TGF-β expression. We found a consistent pattern of expression demonstrating increased levels immediately following laser irradiation and at 14 days, as compared to control wounds in the same patient. While the 14-day increased expression correlated with the increased inflammatory cell infiltrate of predominantly monocyte macrophages that are known potent sources of TGF-β observed in the laser wounds, the early increase (within 15 min) following laser irradiation could not be ascribed to new synthesis (transcription or translation).

TGF-β is a 22-kDa active dimer that is secreted as a small latent

complex (SLC) via its association with latency-associated peptide (LAP). Following cellular secretion, the SLC associates with other matrix molecules such as micro-2-globulin (serum), integrins, and latent TGF-β binding proteins (LTBPs), and is referred to as the large latent complex (LLC). A key limiting step of TGF-β physiological action is the activation of the latent complex involving various physical and chemical modalities such as heat, extreme pH, ionizing radiation, proteases and biophysical forces involving integrin binding.[50-51]

As the early wound undergoing hemostasis has abundant platelets which have abundant LTGF-β, we hypothesized that the early wound milieu will be a rich pool of laser-activatable LTGF-β. To test this hypothesis, we used bovine serum LTGF-β solutions and subjected them to laser irradiation at various fluencies and assessed active TGF-β using an enzyme-linked immunosorbent assay (ELISA). Further, as the activation mechanism involves a conformational change of the latent complex, we also performed a reporter assay to ensure the activated LTGF-β is biologically active. Both assays demonstrated the ability of LLLT to robustly activate LTGF-β. In our experience, fluencies between 1-10 J/cm^2 with a median at 3 J/cm^2 in the near-infrared wavelength (above 800 nm) are optimal in stimulating TGF-β activation and producing PBM in mucosal healing.

ONGOING WORK AND FUTURE DIRECTIONS

Our ongoing current experiments have characterized the precise photomolecular contributions of specific ROS species and the photobiological steps in activation of the LTGF-β. Furthermore, we are applying this novel laser-activated TGF-β mechanism to other biological applications specifically in craniofacial regeneration such as dentinogenesis, mucosal healing, and neuromuscular recovery. Moreover, screening for other photoactivable latent complexes using molecular screening strategies is currently under way.

On a cautionary note, while stimulating a biological process with LLLT with a defined therapeutic end point is clearly useful, we must be cognizant of the possibility of bystander effects where potentially pretransformed cells may be stimulated to progress to tumors. As a key area of future investigation, we are studying such concepts that generating a good healing milieu could counteract the progression of tumor cells, while the persistence of a chronic wound environment is detrimental and promotes tumor growth. Among the beneficial healing milieu is the key role for reducing inflammation. Thus, given the direct contribution of inflammation to cancer in some scenarios, the beneficial effects of PBM could also be indirectly limiting for neoplastic processes.

In conclusion, LLLT-mediated PBM is a useful clinical tool and as we uncover the precise mechanisms governing its biological effects, a robust, consistent, and efficacious clinical therapeutic regimen should be possible in the near future.

AUTHOR BIOGRAPHY

Dr. Praveen R. Arany received his BDS in 1999 from Karnatak University, India and his MDS in Oral and Maxillofacial Pathology in 2001 from Rajiv Gandhi University of Health Sciences, India. He then completed two postdoctoral fellowships at the Indian Institute of Science, Bangalore, India and the National Cancer Institute (NCI) at the National Institutes of Health (NIH), Bethesda, Md. Dr. Arany also has a Certificate in Clinical Research from the NIH and currently is completing a joint Residency-PhD program in Dental Medicine at Harvard University. He has several awards to his credit including an NCI Director's Innovation award, an NIH MERIT award, Wound Healing Society's Young Investigator award, Pulp Biology and Regeneration Award from the International Association for Dental Research, and, recently, the Dr. Eugene M. Seidner Student Scholarship award from ALD. He has more than 25 publications including 4 textbook chapters to his credit and is an invited speaker in national and international forums. He is on the editorial board and reviews for several scientific laser journals and is the current scientific chair for the North American Association for Laser Therapy (NAALT) 2011. Dr. Arany may be contacted via e-mail at arany@fas.harvard.edu or praveenarany@gmail.com.

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