Laser Biostimulation: Review and Hypothesis

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Introduction

SURGICAL lasers such as the carbon dioxide, argon and neodymium YAG, focus intense light into a precise target area, creating very high power densities. Their primary mode of interaction with living tissue is thermal, causing denaturation of proteins, melting, coagulation and vaporization. Another class of light/tissue interactions occurring at low power densities produce much different effects. These tissue reactions, termed "photochemical," are essentially non-thermal.

The reception and transduction of light in the retina is a familiar example of photochemistry. The initial event in vision occurs when a quantum of light is absorbed by the visual pigment, rhodopsin (1,2). Rhodopsin is a protein, opsin, plus the chromophore, retinal. The additional energy provided by the photon triggers a conformation change in retinal (isomerization of 11-cis to 11-trans) followed by a cascade of reactions resulting in an electrical event in the receptor cell (3).

A second example of photochemistry is the process of photosynthesis used by plants and bacteria. The initial event in photosynthesis is the absorption of several quanta of light by the chloroplast pigments (4), chlorophyll a and b. In this process electromagnetic energy is ultimately converted into phosphodiester bonds in ATP, a convenient form of energy used throughout the cell.

Photodynamic therapy (PDT) for the treatment of cancer is another example of a photochemical, laser/tissue interaction. In this case the chromophore is the tumor-localizing drug, hematoporphyrin derivative (HPD). HPD is injected in the blood stream and becomes concentrated in malignant tissue through the processes of selective uptake and selective retention (5-7). The exact sequence of events that occurs following the absorption of light by HPD is still uncertain, but it is fairly well established that an energy transfer occurs from the HPD triplet state to molecular oxygen. This, in turn, creates singlet molecular oxygen that attacks and destroys the host tumor.

These examples are provided as a context for understanding the photochemical reactions that might occur in laser biostimulation. Here, however, the light-absorbing chromophores have not been identified, so the subsequent biochemical reactions have, also, not been specified. As a result, rather than rely on a mechanistic definition of biostimulation, what is popular is an operational definition provided by its clinical applications.

In 1984, The National Center for Devices and Radiological Health of the FDA issued a cautionary statement on laser biostimulation (8) as an "unproved" medical treatment that was claimed to reduce pain, accelerate wound healing and provide non-surgical facelifts. Although there was support for pain control and wound healing published outside the United States, there was (and still is) no documented evidence that low-level laser energy was effective for non-surgical facelifts. The FDA expressed concern because laser biostimulation devices were being promoted without the requisite studies on efficacy and safety. In addition, the mechanism of action was unknown.

Laser biostimulation currently enjoys widespread clinical popularity in Europe, Canada, China, and Japan and there is now an extensive literature on effects of low-level laser irradiation on cell and bacteria cultures, in animal experiments, and from clinical studies. Although it is difficult to determine how many studies have yielded no effect (negative data is usually not published), most published results point to a safe and positive medical outcome. Even though there are numerous reports of its effectiveness the large majority of these studies have been poorly controlled, or have had no controls. However, some of the clinical trials have been well designed and a few have studied similar disease processes, allowing comparison of results.

The clinical application of laser biostimulation is extremely controversial, especially in the United States. The purpose of this review is not to evaluate the quality of the published clinical studies. The clinical material is reviewed to provide a context for understanding the basic scientific and pre-clinical animal studies. The latter type of studies, mostly free from hype and quackery, provide evidence that laser biostimulation is a fact, not fiction.

The reputation of biostimulation has also suffered from a lack of widespread basic scientific inquiry such as is enjoyed by vision, photosynthesis and PDT. However, from the scientific information, most of it published within the past few years, we can now hypothesize a reasonable explanation of the mechanism of action of laser biostimulation.

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Editor's Note:

This paper was accepted for publication through a peer review process, recently initiated for submissions to the forthcoming Journal of Laser Applications.

The purported benefits of laser biostimulation continue to be a topic of controversy. Our reviewers commented on the need to recognize, also, that there is controversy regarding the interpretation of many of the clinical studies. In part, this is due to faulty design of the studies, and premature and/or unjustified claims of benefits achieved.

The author states: "... my purpose is to provide a clinical context and then examine the scientific literature. I propose a mechanism of action... which I believe to be a working model."

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Research in Wound Healing

Laser biostimulation was pioneered at the Postgraduate Medical University in Budapest, Hungary, by Dr. Endre Mester (9). Clinical trials began on non-healing ulcers in the early 1970s and have continued until literally thousands of people have been treated under a constant treatment protocol. An excellent review of their scientific work and clinical trials in wound healing is soon to be published (10).

In addition to the treatment of non-healing cutaneous wounds (11-17) are reports of biostimulation being effective in the acceleration of bone repair following fracture or radiation-induced necrosis (18-22). An interesting series of experiments have indicated that low-level laser irradiation may provide "protection" of peripheral and central nerve function following crush injury (23-27), although the results are still quite preliminary.

This list of applications is not exhaustive (pain control is discussed below). Several good commentaries on biostimulation for wound healing are available (28, 29) so a thorough literature review is not necessary here. Suffice it to say that most of the work has been done on a variety of animal models and humans, with various laser systems and treatment protocols. Some papers report no effects (30-32), whereas other reports border on the miraculous. The literature is disorganized and many studies are haphazard. Almost all publications lack sufficient information especially in the reporting of exposure parameters. A few general observations relative to the scientific basis can be gleaned from the extensive, if not coherent, literature.

A typical treatment protocol involves irradiation of the affected site with low-power density laser light (<200 mW/cm^2) for a duration that produces a total energy density (light dose) around 0.5-4 J/cm^2. Since the effects are apparently cumulative, treatment is repeated at intervals from daily to weekly, usually 2-3 times per week. Endpoints such as wound closure, pain relief, etc., are often achieved within 1-3 months. Lasers with outputs in the red (HeNe, 628.5 nm) and near-infrared (GaAlAs, 800-900 nm) seem to provide the best results.

There appears to be a significant placebo effect, a narrow dose/effectiveness window, and an inhibitory effect at higher light doses. These characteristics are diagrammed in Figure 1. The placebo effect is undoubtedly more important in pain control than in wound healing because of the magnitude of the psychological component in the perception of pain. Biostimulation occurs between about 0.05-10 J/cm^2. Lower values are reported in cell culture studies (33-36) than *in vivo*, probably because of the attenuation of light as it passes through tissue. The magic number of "4 J/cm^2" was arrived at empirically by Mester, et al (37). Energy densities above 10-50 J/cm^2 have an opposite effect on tissue metabolism termed, "bioinhibition." Mester, et al (36) identify this as an example of the Arndt-Schultz law, "low energy densities are stimulative while high energy densities are inhibitory." Bioinhibition has found clinical application in the treatment of keloids and hypertrophic scars (38-40).

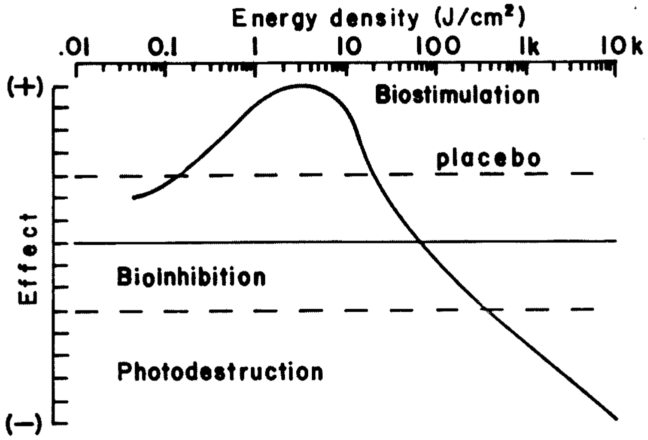

FIGURE 1. Schematic dose/response curve for low-level laser irradiation. Details of this function undoubtedly vary with wavelength.

There seems to be a conflict between the results from human trials and those from animal studies. In human studies reporting positive effects, laser biostimulation accelerates closure or provides healing of non-healing wounds. (Non-healing wounds are those that do not respond to conventional therapies.) In several animal models experimental wound closure is not more rapid with laser treatment than without (41-43), but increased tensile strength and collagen concentrations are found (41,42,44-49). It may be speculated that normal healing occurs in the animal experiments, whereas the healing process is often compromised in human studies (e.g., diabetic ulcers). This interpretation is supported by fibroblast culture studies (50). Cultures with initial high rates of collagen synthesis showed little or no effect following laser irradiation, but cultures that had low rates of collagen production showed the greatest increases following laser irradiation. The tentative conclusion here is that laser biostimulation will not improve the healing process (or increase cell metabolism) much beyond what is normal.

Research in Pain Control

Clinical applications of biostimulation for pain control seem to have become more popular than applications for wound healing. A good summary of the clinical literature in pain control is provided by Basford (28). Cases of muscular pain, neuropathic pain and pain associated with arthritis have been reported. Most of the published reports seem to be on treatment of pain from osteo- and rheumatoid arthritis (51-63).

Treatment protocols for pain are similar to those described for wound healing. There is an important difference. In wound healing the surface is irradiated, but in pain control light must penetrate more deeply. Often, a pencil-like probe containing a fiber optic is placed in contact with the skin overlying the affected joint and the light is transmitted through the epidermis to scatter within the deep layers. In this case energy density at the surface is probably not a parameter that is relevant to the underlying process. Rather, flux density surrounding the joint in J/cm^3 should be considered in dosimetry for pain control.

In addition to direct treatment of the painful site is the practice of laser acupuncture (63-69). This procedure, although reported effective and in common use outside of the U.S., confounds one mystery with another. Apparently acupuncture can be achieved by stimulation of acupoints mechanically with needles, electrically with transcutaneous electrical nerve stimulation (T.E.N.S.), or photonically using laser light. Although the mechanism of action of acupuncture is still uncertain, these three techniques may have a similar mechanism: the release of central nervous system endogenous opiates (61,70). Some therapists use direct irradiation (e.g., 52), some use only laser acupuncture (69), and some use a combination (63).

The pain associated with rheumatoid arthritis may result from several pathological events. Certainly the edema and inflammation contribute. In addition, there is the suggestion of local sensitization of peripheral C fibers and A-delta fibers that transmit pain signals into the spinal cord (71-74). Sensitization is manifest as a chronic change in resting membrane potential toward firing threshold (depolarization).

Experiments measuring pain thresholds in animals following laser biostimulation have not yet been reported. However, both animal and human studies have identified an increase in compound action-potential latency of sensory peripheral nerve following low-level laser irradiation of the distal end. Decreased conduction velocity of nerve bundles is inferred from increases in compound action potential latencies in sciatic nerve of rats (75,76) and radial nerve in humans (77); although one study, not reporting the laser energy used, found no effect (78). Increased compound action-potential latencies can also indicate a greater dissynchrony of firing caused by changes in firing thresholds.

Hyperpolarization of peripheral nerves changes the threshold of membrane excitability and could lead to a decrease in conduction velocity. A hyperpolarizing change in resting membrane potential also reverses the process of peripheral nerve sensitization, proposed above as a mechanism for producing pain. It is therefore important to measure whether or not low-level laser irradiation affects nerve membrane resting potentials.

Single neuron recordings of the electrophysiological consequences of laser irradiation have demonstrated an effect on firing patterns (79,80) and laser-induced neurotransmitter release (81). Unfortunately, these studies employed energy densities well above those used in biostimulation, so that the precise effect of low-level laser irradiation is still unknown.

Research in Biochemical Mechanisms

Until 1984, most biochemical and ultrastructural studies of laser/cell interactions used high-power densities (10^3 to 10^8 W/cm^2) and high-energy pulses to discover effects of irradiation (79-85). However, some very interesting facts emerged from these studies. The primary subcellular sites of light absorption are the mitochondria. Within mitochondrial membrane the principle chromophores seem to be the cytochromes (35,80, 82,83,85,86). The absorption of light alters transmembrane potentials (79,80,85,86) and stimulates synthesis of ATP (35,83,86).

An action spectrum is a graphic description of the magnitude or sensitivity of an effect shown as a function of wavelength. These spectra often reveal characteristics of the underlying molecular processes. For example, vision spectral sensitivity curves of color-blind subjects reveal the operation of only two of the three color-vision pigments (1). Karu (35) has provided detailed action spectra for biostimulation of the rate of DNA synthesis in HeLa cells in culture, and for growth of bacteria and yeast colonies. The spectra show peaks in the blue (404 and 454 nm), red (620 nm) and near infra-red (760 and 830 nm). These peaks correspond to some of the peaks of cytochrome absorption.

There is some doubt that the coherence of laser light plays a significant role in biostimulation (35,87). This is understandable since the maintenance of any coherence within the highly scattering medium of biological tissues is unlikely. However, it has been suggested that monochromaticity is important since evolution may have cleverly arranged for closely adjacent colors to have opposite, counterbalancing effects (35). Lasers, especially low-cost HeNe and diode lasers, do provide a very convenient source of intense monochromatic light.

Action Mechanism of Biostimulation

It is possible to propose a tentative mechanism that is consistent with the information presented so far. This working model has some plausibility, it predicts certain clinical outcomes, and most importantly, it is testable with standard scientific methodology.

Consider the example of photochemical reactions in photosynthesis. The action spectrum of photosynthesis represents the combined absorption spectra of chlorophyll a, chlorophyll b and b-carotene (88), part of the light harvesting complex (LHC) embedded in the chloroplast membrane (89-91). Absorption of a photon excites an electron in the chlorophyll and initiates the movement of the electron from one end of the LHC to the other. Thus, light absorption by the chlorophyll molecules is translated into electrochemical energy through electron transport across the chloroplast membrane. This light-driven separation of charge operates the "proton pump." Proton pump energy is used for the phosphorylation of ATP from ADP. ATP is then used by the plant to do all those biochemical things that plants do. Many types of bacteria also use photosynthesis to drive metabolism. The purple bacteria, rhodopseudomonia, use a different chromophore known as bacterio-rhodopsin although the ATP-generating chemistry is quite similar to that of chloroplasts (92, 93).

It is suggested that biostimulation is very similar to photosynthesis. This similarity becomes more apparent if we consider two other popular concepts: (1) the "endosymbiotic hypothesis," concerning cellular evolution, and (2) the "chemiosmosis theory," basic to modern biochemistry.

A few billion years ago, the evolution of single cell organisms is thought to have branched into three separate paths. Two major branches formed eukaryotic cells (plants, animals, fungi, etc.) and prokaryotic cells (bacteria). Then, according to the "endosymbiotic hypothesis," about 1.5 billion years ago a purple bacteria formed an evolutionarily successful relationship with a primitive form of nucleated cells (94,95). These bacteria flourished within the protective environment of the host cell's cytoplasm, and the host benefitted from an increased efficiency in energy production. Thus, the ancestors of the mitochondria found today in every human cell are thought to have been these photosynthesizing bacteria. Based upon this hypothesis it can be suggested that this primitive photosynthetic mechanism is still intact in mitochondria and functions in biostimulation.

The "chemiosmosis theory" is based on the universal principle that concentration gradients across membranes and the phosphodiester bonds in ATP are interconvertible forms of storing energy (96-99). All living cells require energy for growth and metabolism, usually supplied by the hydrolysis of phosphate bonds in adenosine triphosphate. ATP must have existed in the earliest life forms since it is found in all types of organisms: bacteria, plants and animals. Consequently, there is a great similarity in how ATP is generated by both photosynthesis and mitochondrial aerobic oxidation. Transmembrane movement of protons down their electrochemical gradient is coupled in both systems to the phosphorylation of ATP from ADP + phosphate. In photosynthesis this proton pump is powered by absorbed light energy. In animal mitochondria electron transport is powered by the oxidation of metabolic products of sugars and fatty acids. In both cases the electron transport system separates charge to establish the proton concentration gradient used in the synthesis of ATP.

The biochemical data on biostimulation reviewed above suggest that the cytochromes in animal mitochondria may be the primary light absorbers. The absorbed energy would enter the electron transport chain, work to separate charge across the mitochondrial membrane and power the synthesis of ATP. In fact, chlorophyll and cytochrome molecules are structurally similar, both have a metalated, conjugated porphyrin ring that is an efficient light absorber (100). Therefore, several independent lines of evidence point to the possibility that metabolism can be driven by the absorption of light by the cytochromes in mitochondria. In other words, biostimulation may locally activate a primitive photosynthetic mechanism.

It follows that in the various forms of laser biostimulation, light energy may be converted into usable chemical energy in the form of phosphodiester bonds. This "extra" energy is used to produce a variety of metabolic consequences depending on the tissue irradiated. For fibroblasts the result is increased collagen production. For nerve cells the result is increased activation of the ATP-dependent NA-K pump. In the latter case this would increase the potential difference across the cell membrane (hyperpolarization) moving the resting potential further from firing threshold, thus, decreasing neuronal sensitivity.

Conclusions

A great deal of controversy surrounds laser biostimulation. There are three major reasons for skepticism: (1) false claims and charlatanism, (2) efficacy has not yet been demonstrated unequivocally with properly controlled clinical trials, and (3) the basic mechanisms are poorly understood. There is certainly not a definitive set of optimum treatment parameters for biostimulation. For example, wavelength is often chosen on practical considerations, i.e., what type of laser is available? Karu (35) has published the only known action spectra for biostimulation, which are limited to cell cultures. The clinical action spectra may be quite different, due to differences in absorption characteristics between human and bacteria chromophores and light scattering. The best wavelength for accelerating wound healing (blue?) is probably different than for pain control (red-I,R,), due to the wavelength dependence of light transmission through living tissues. Without clinical or animal action spectra of biostimulation efficacy we cannot definitively answer the question: what is the best laser?

If 4 J/cm^2 is the optimum energy density, what is the optimum time for delivering this dose? Assuming that we maintain power density below 400 W/cm^2 (avoiding hyperthermia), then delivery time must be at least 2.5 seconds. But beyond this we do not know how dose rate influences the outcome of biostimulation. Is the optimum dose the same for wound healing as for pain control? Probably not. How does the treatment of multiple sites influence the outcome? There is also the clinical question of the proper treatment schedule (every hour, 1/day, 3/week, 1/week?). One report in an animal model suggests that every other day is better than every day in terms of wound closure (101; see also Table III, ref. 32).

Each application where biostimulation may be an important adjunctive therapy; pain control, non-healing skin ulcers, bone repair, peripheral nerve repair, skin grafts and transplants, may have a unique set of treatment parameters. It is obvious that all of these variables cannot be defined empirically from human clinical trials.

Perhaps the haphazard, disorganized nature of the clinical biostimulation literature results, in part, from the lack of a working hypothesis on the underlying mechanisms. The missing and essential item of information is the identification of the chromophore. Data from a number of papers (35,80,82,83,85,86) suggest that the cytochromes may be the "biostim molecules," but without direct confirmation. Other scientific deficiencies include good animal models for non-healing wounds and pain control, essential for establishing the parametric studies needed to define optimum treatment protocols. Basic questions about the mechanisms for pain control could be answered with careful extracellular and intracellular recordings of neuronal firing patterns and transmembrane currents in response to low-level monochromatic irradiation.

The action mechanism "story" outlined in this paper is offered as a possible set of organizing principles to guide clinical and basic research. The suggestions made are presented as testable and, thus, hypotheses subject to disproof.

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