

Optimizing Laser Phototherapy

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Therapeutic Laser Industry Overview

As has been the case with technology throughout history, this field was pioneered by engineers, not academics, and it's a good thing. After all, if we waited until we understood why something works before we used it, we would be in the stone-age, literally (people were eating vegetables before they knew why plants grew and warming their bodies with fire before they knew about combustion). This young field has therefore suffered from the zeal of its boosters; people had found a way to make money, and to their defense, improve patient quality of life, and so were much less interested in learning *why* this therapy worked. In this last decade, the research community has been catching up and we have ever-growing insight into the mechanisms of this highly successful field.

Differentiating What We “Know”

Because this field is so young, there are still huge gaps in our knowledge base, both in the physical properties of the individual lasers, as well as in the biological implications thereof. There is now, however, a well-established research community dedicated to the general study and optimization of the biological effects of laser phototherapy. Experimentation falls into two major categories: *in vitro* and *in vivo* studies, each of which is necessary, but extrapolating results from one to implications in the other is difficult and often misleading.

In Vitro Studies

Microscopic studies on laser interaction with biological material are invaluable. They give us the ability to precisely control the cellular environment and completely isolate a huge range of absorption mechanisms en route to a better understanding of not only the governing dynamics behind the macroscopic success of phototherapy, but also techniques to optimize its delivery and efficacy. Mountains of research have been done on individual bacteria and mammalian cells and on monolayers of such cells in petri dishes. From these, along with concurrent work on molecular biology, we have very clear picture of where light is absorbed in cells and which processes these interactions catalyze.

There is an important caveat, however, which resonates throughout the entire biological community: the reaction of a macroscopic matrix of cells that form tissue is *NOT* the sum of the reactions of each of the individual cells. One of the great mysteries of biology involves the complexity of cell-cell signaling and the ubiquity of bystander effects. A prime example of this intrinsic communication is in radiation oncology where researchers have used X-ray needles (microscopically narrow beams of x-rays) to irradiate individual cells growing in a monolayer. Amazingly, cells far away from the irradiated region somehow received information from the irradiated cells and underwent apoptosis (programmed cell death) in a way that is characteristic of cells that absorbed the ionizing radiation (even though they didn't). Accordingly, we have to narrow the scope of individual cell and single cell monolayer studies to the search for absorption sites and the cellular functions affected by these sites, and stay away from making broader tissue-scale generalizations.

***In Vivo* Studies**

Studies on human patients, and to a lesser extent laboratory animals, are often the most convincing to potential commercial users (i.e. chiropractors, podiatrists, veterinarians, dermatologists, etc.) of therapeutic lasers, and with good reason. Proven functionality on the proposed client base is a very marketable result. They are, by nature though, expensive and time consuming, and for that reason are usually biased to keep the number of possible outcomes narrow and manageable. Also, since they are typically carried out with commercial laser setups that require purchase, studies that compare multiple types (brands) of lasers to each other and to control groups are rare. Far more often, a study compares the effects of a single laser to a control group; the slightly more robust trials at least vary a parameter or two (e.g. power density, treatment length, pulse frequency, etc.).

Results from these types of studies yield conclusions *that* a particular treatment works, but not why or even if this particular treatment is most efficient. For example, a case study showing the elimination of a toe fungus with pictures before and after 6 weeks of therapy is encouraging in that it works, but does not tell you that the use of a different kind of laser could have provided the same results in 4 weeks.

Still, clinical trials are indeed necessary and continue to give important perspective into the macroscopic effects of phototherapy. The increasing popularity and success of this field is almost exclusively attributed to the number and breadth of clinical applications. To understand the mechanisms of action, though, we must combine our studies with a closer look into the cellular, and deeper still, molecular interactions of laser radiation with biological material. We can then use both micro- and macroscopic results to guide the search for the most efficient therapeutic techniques.

The Well-Oiled Human Machine

By far the most obvious and fortunate conclusion we have been able to extract from *in vivo* studies (not only with respect to laser phototherapy) is that our immune system is capable of handling an extraordinary range of pathologies. The time scale and degree to which our cells can react and combat these contaminants is the subject of much study,

but it is clear both that lasers do stimulate the immune system and that the restoration of healthy function continues well after the initial irradiation. The amount of healing done during the minutes of laser irradiation is minuscule compared to the time it takes to relieve the body of disease or infection. This leads to one very important piece of information: the body does most of the work itself and so the target for an effective laser treatment is *NOT* the pathology itself, but rather to stimulate the appropriate cell compartments that lead to the body's natural repair mechanisms. Basically, we want to stimulate the cell's metabolism (i.e. its ability to use oxygen to create energy).

Bacteria, on the Other Hand

There are about 1000 different types of bacteria commonly present in the human body most of which reside either on the skin, or in the digestive tract. Of these, only about 10% are maintainable in cell culture and able to be studied. Some are beneficial (e.g. those that aid in digestion of food) others pathological. With this wide variety of species, never-mind their different functions and chemical signatures, it is prohibitively difficult to target any individual candidate or even to make the generalization that these candidates are more abundant than any other with respect to a particular pathology. Instead we can capitalize on one common feature in most bacteria: they do not like oxygen. Most bacteria are anaerobes that proliferate and metabolize much better in the absence of oxygen. Fortunately, this is in direct contradiction with the way our cells flourish and so stimulating the oxygen intake and conversion process will simultaneously help our healthy cells and inhibit bacteria.

Mechanisms

Identifying Targets

The most fundamental thing to keep in mind is that the cell (and the body as a whole) is comprised of more than 80% water. The variation in water content between different kinds of cells (with the exception of bone cells) is negligible and so laser therapy as a whole is highly non-selective. Cells do, however, contain some heavier elements that can act as a contrast agent against water, and which can therefore be targeted with laser radiation; the most relevant examples are iron and copper. Not surprisingly, these elements are the ones that exist at the core of the two most important photoacceptors in the body: hemoglobin at the core of blood cells and cytochrome c oxidase in the mitochondria. By and large these complexes are the principle absorbers of mammalian tissue by light in the near infrared (NIR) range of the electromagnetic spectrum (other than melanin in the skin). As such, and before any attention to their function, the characterization of absorption of these complexes was of paramount importance, and the subject of much study. Action spectra (i.e. the dependence of wavelength on absorption) have been generated for these (and other) targets *in vitro* and the peaks have been isolated and correlated with the biologically state of these complexes (see section "Clinical Functionality").

Metabolic Action

The action spectra tells us where in the spectrum and at what rate laser radiation is absorbed by these chromophores, but we must address the biology of the cell to understand the subsequent chain of events that lead to a beneficial, curative result. As discussed earlier, the central goal is to stimulate the cell (and ultimately, the body) to perform its natural functions, but at an enhanced rate. These natural functions are not only extremely numerous (ranging from protein synthesis to enzyme secretion, from cell signaling to physical movement) but also highly cell-type dependent. Any attempt to directly target one of the multitude and variety of these specific enzymes is difficult, and fundamentally unnecessary. If instead, the metabolism, specifically the respiratory chain, can be stimulated, the cell will enhance the functionality of *all* of its natural processes.

Fortunately, both hemoglobin and cytochrome c oxidase are involved in cell metabolism and their roles in the respiration chain are linked. Hemoglobin is the molecule, at the core of red blood cells, that transports oxygen through the body to the cells. When it reaches the cell it has to be de-oxygenated or “reduced”. The oxygen is then passed through the cell membranes and into the mitochondria where it is processed by a series of enzymes, the last of which is cytochrome c oxidase. Here the oxygen is again “reduced” as it is converted into water; this reaction is the stimulus for the enzyme ATP synthase to create ATP, the source of chemical energy in cells. This is the reason we need oxygen, slightly more in depth than “to breathe”.

Optimizing Efficiency

Zooming out to the big picture, hemoglobin carries the oxygen through the blood from the lungs to the cells. It has to be reduced and the oxygen flows through the respiratory chain to the terminal enzyme, cytochrome c oxidase, which then reduces again to create energy for the cell. Think of the hemoglobin as the faucet that governs the rate at which oxygen flows *into* the cell and cytochrome c oxidase as the drain that determines the rate at which oxygen can *exit* the cell in the form of ATP (energy). To optimize efficiency of the flow of oxygen through the respiratory process, the most appropriate course of action would be to open both the faucet and drain as wide as possible (opening one without the other would not increase the overall throughput); that is, stimulate the amount of hemoglobin that reaches the cell, the rate at which it reduces its oxygen, and then the rate at which the cell can process that oxygen and output energy. The goal then is to increase local blood circulation, stimulate the reduction of hemoglobin, then stimulate both the reduction and immediate re-oxygenation of cytochrome c oxidase so the process can start again.

Clinical Functionality

Circulation

Recall the first goal of an effective therapy was to increase the amount of oxygen available for the cell to process. This means increasing blood circulation since the

hemoglobin in red blood cells are the transporters of oxygen from the lungs to the cells. On the macroscopic scale, this relies on increasing the heart rate, which in turn slightly increases body (and blood) temperature. This is why exercise is good therapy for almost any ailment; increasing blood flow increases metabolism and stimulates the immune system. Locally around a wound, however, topographical heating does very little, resulting in neither an increase in circulation nor metabolism. This type of thermal effect is *not* the mechanism for laser stimulation of circulation. Laser irradiation instead creates local temperature gradients; that is, temperature differences on the molecular level that create potentials along which blood cells are more likely to flow. The stronger and more numerous the gradients, the more local circulation of oxygen can be stimulated.

What is the most efficient way to cause these temperature fluctuation? Recall that the cell is more than 80% water. If you can target the absorption of water by a particular wavelength of radiation, you can cause local resonances that reinforce themselves. In the entire NIR region (i.e. from 700-1000 nm) the strongest and most distinct peak in absorption is at 965 nm; the right side of Figure 1 shows the absorption spectrum of brain tissue in the NIR.

Hemoglobin Deoxygenation

Once the increased circulation gets the blood to the cell, the hemoglobin that carry the oxygen in the blood have to drop off their oxygen supply. Oxygenated and deoxygenated hemoglobin have very distinct signatures in the NIR. We are not concerned with the process of re-oxygenating the hemoglobin, because this occurs in the lungs. Instead we are interested in the absorption spectrum of oxygenated hemoglobin (HbO₂) whose deoxygenation can be stimulated by the absorption of a photon of radiation. Figure 1 shows this rather broad peak that covers the higher end of the NIR.

Cytochrome c Oxidase Redox

As discussed earlier, the terminal enzyme in the respiratory chain of a cell, cytochrome c oxidase, is the principle absorber of radiation in the entire cell and governs the rate at which oxygen is processed into ATP. Unlike the one-way deoxygenation of hemoglobin, cytochrome receives and delivers its oxygen in cycles within the cell and so we need to stimulate both processes in order to maximize efficiency. It turns out that laser irradiation does both, depending on the oxidation state of the enzyme. When deoxygenated, laser irradiation will stimulate oxygenation, and vice versa [1]. This effect has resounding implications and is thought to be the universal validation of laser therapy. The different oxygenation states of this enzyme have peaks throughout the visible-NIR spectrum, which is why virtually all wavelengths used have shown to be useful.

Laser phototherapy with wavelengths throughout the NIR spectrum enhances cellular metabolism, but there exists a peak in the absorption spectrum that can maximize this effect. Figure 1 shows the difference spectrum in the absorption of oxygenated vs. deoxygenated cytochrome. Remember, when the enzyme is either fully oxygenated or fully deoxygenated, irradiation will push the cycle along in the right direction, so

we want to stimulate the process at both endpoints. The peak in the difference spectrum reflects the wavelength at which laser irradiation will have the greatest effect to change the oxygenation state, which will subsequently turn the wheels on the cellular metabolism most efficiently. This is analogous to firing the spark plugs at the exact time in the engine cycle to get the maximum effect.

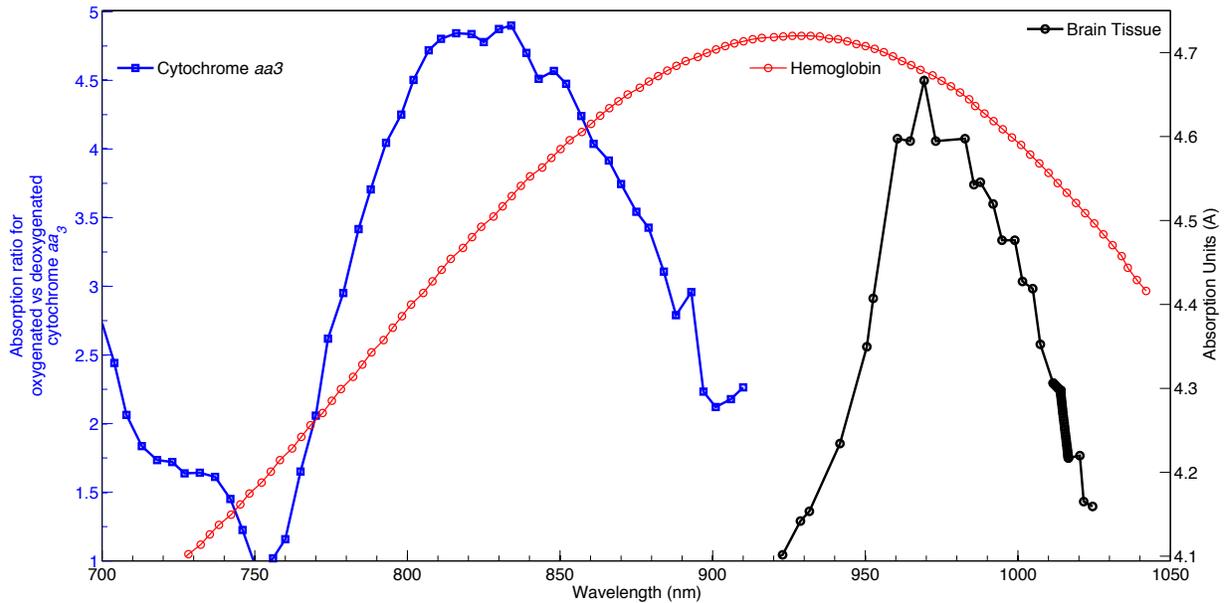


Figure 1: Absorption spectra of cytochrome (left axis), oxygenated hemoglobin (a.u.), and brain tissue (right axis). Data re-digitized from [2].

Take Home Message

FACT: Laser phototherapy, if administered by someone trained in the art, is beneficial in almost all of its forms and has no adverse side effects.

The differences between commercially available laser units lie solely in the wavelength, power density, pulse modulation, and aesthetics. From these parameters, you can derive the penetration depth, dose distribution, treatment time, and the estimated biological effect. There is *NOT* a “magic” wavelength or setting that is the cure for a disease, and to claim otherwise (as many distributors or salesmen do) is irresponsible. There are, however, certain operating regimes that give better results than others and are more effective for particular symptoms. The select few modalities that have been specifically designed to isolate and capitalize on a fundamental therapeutic mechanism,

have continually proved successful in the clinic. And since the primary mechanism of action is the stimulation of the body's natural anti-pathological immune system, the range of symptoms for which this treatment modality is useful knows no bound.

References

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