REVIEW ARTICLE

LASER AND LED PHOTOBIOLOGY

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Low Level Light Therapy, or LLLT, is a very important new area of photomedicine. However, there are a number of reasons why LLLT has not been accepted into the main stream of science and medicine, and I will discuss a few of them below. Let me add, however, that I know from personal experience that LLLT works on wound healing, and carpal tunnel syndrome, when done properly.
I have been trying for over 40 years to teach photobiology to laser people, and more recently, to LED people as well. At a laser meeting in the 1970s, at the height of the feeling that lasers were magical, I made a slide to demonstrate the biological effect of a laser. It showed a man dropping a big laser on his foot, and yelling OUCH. I said that this is the ONLY type of biological effect that a laser can have on a person. A laser is an expensive flashlight, and it is the light produced BY the laser that has a chance of producing a biological effect, assuming that it is of the correct wavelength and output, etc.
Because so many bad papers have been published on LLLT, it has not achieved the universal acceptance that that it deserves. There are two main reasons for bad papers on LLLT; one is the lack of proper scientific training by the authors, and the other is their lack of knowledge of photobiology. I will give you some examples of very bad science.

At an LLLT Congress some years ago, the speaker proclaimed that LLLT was effective at reducing high blood pressure. In the so-called clinical trial, the patient came in and had his blood pressure taken. Then various areas around the patient's neck were irradiated with a laser, whose wavelength I don't remember. Then they measured the patient's blood pressure again, and low and behold, the blood pressure was lower. Was this another magical success for LLLT? NO!

I asked the speaker if he had also tested the patient's blood pressure 30 minutes after the therapy, or after 1 hour, or 2 hours, and he said no. Then I told him my story. While preparing for my trip to that Congress, I was running about the drug store picking up toothpaste, etc., and I saw a new blood pressure machine, and decided to try it. My blood pressure was way above normal. After a few moments of sitting and reading the signs on the machine, I decided to test my blood pressure again, and low and behold it was normal, even without laser treatment. Sitting down in a chair for a few moments worked magic.

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The problems mentioned for the blood pressure experiment are by no means exceptional. Numerous papers in the literature report that an experiment was carried out for a certain period of time, and with each irradiation there was improvement. What would happen if the treatments were carried out longer? Would the beneficial response continue, or would it reverse and become detrimental with continued therapy? How about checking the patient a week later? Would the result still be positive, or was the treatment effect only temporary?

Here is another example of bad science and bad photobiology from a paper on delaying optic nerve degeneration by low-energy laser irradiation. [1)] The authors used non-coherent 904 nm radiation, and coherent 633 nm radiation. They reported that the non-coherent light adversely affected the injured nerves, while the coherent light was effective. They concluded that coherence is important for LLLT.

Unfortunately, this paper proves nothing about coherence. In the first place, you can't run a proper scientific experiment with two major variables, wavelength and coherence. They varied the wavelength, but they did

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not understand photochemistry. At the same time they also varied coherence, but they did not understand physics.

Nothing was mentioned about heat during the treatment of the optic nerve, so we do not know if the authors made sure that they were not cooking the nerve with the 904 nm LED, and that is why 904 nm light was not effective? The authors point out that the energy densities for the 904 nm LED was much lower (25.5 and 16.9 J/cm^2) than those of the 633 nm laser (132.7 and 39.8 J/cm^2). Could this be the reason for the failure of the 904 nm radiation?

Also, the light treatment only delayed the death of the nerve, it did not reverse the damage. So what is the point of the publication? It is not a success story for LLLT. It is bad science. This paper should not have been published.

Unfortunately, this paper is cited in "The Laser Therapy Handbook" [2; p.13] as proving that coherence is very important in LLLT.

Enwemeka et al. [3,4] have published two papers about controlling infections of methicillin-resistant *Staphylococcus aureus* with blue light. This is certainly of great interest, but in each paper these authors proclaim with wonderment that the survival curves are not linear.

When people enter a new field, such as radiation biology, they should do their homework. Radiation biologists have been producing survival curves for many decades. Of course, radiation survival curves are NOT linear, and no radiation biologist would plot data on linear graph paper. They use semi-log plots, because these plots provide a lot of information about the organism being studied.

Figure 1 shows a survival curve on *Staphylococcus aureus*, which was irradiated with blue light, and plotted with linear coordinates. [4] All that one can conclude from this plot is that cells were killed.

However, if you plot the same data on semi-log paper, you obtain much more useful information **(Figure 2)**. You immediately see that 30% of the cells are much more sensitive than the remaining 70%, and both curves show one-hit kinetics. But why are there two populations of cells with markedly different sensitivities to blue light?

Since the authors do not mention how the bacteria were grown, we do not know if part of the cells were in a different phase of the cell cycle, e.g., stationary phase, and this might be why their radiation sensitivity was different. Without a proper description of how the authors performed this experiment, we will never know the answer.

There is more information on this paper from a Letter to the Editor by Sommer and Zhu [5] entitled "Phototherapy Miracles In A Nutshell". They say that "The fact that light used in phototherapy can perform miracles was recently impressively demonstrated by Enwemeka et al. [4] by killing methicillin-resistant *Staphylococcus aureus* in 24 h with intensive (30m

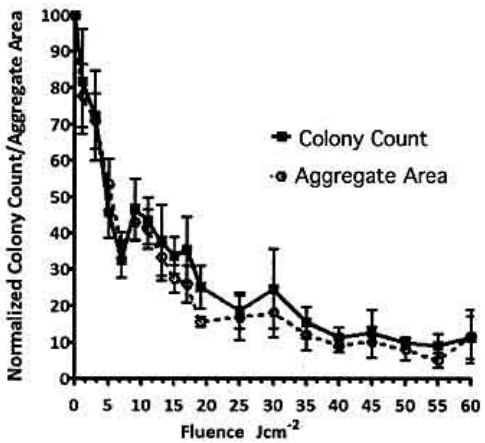

Figure 1. Effect of 470 nm light on colony count and aggregate colony area of the IS-853 strain of methacilin-resistant *Staphylococcus aureus* (modified from 4).

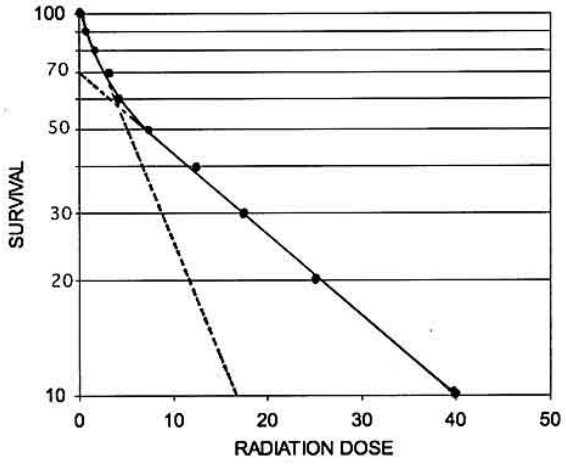

Figure 2. The data in Figure 1 are replotted on semi-log paper, resulting in more useable information (see text).

W/cm^2) blue light."

The Enwemeka paper does NOT describe a miracle in phototherapy. It is a bad paper on radiation microbiology. The authors only killed the cells to 10% survival. This is not enough killing to cure an infection. One would need to kill to at least to 0.01%, and maybe that is not enough.

If you extrapolate the curve in **Figure 2** to 0.01% survival, it would require 185 J/cm^2 of 470 nm light. What would be the consequence of this very high dose of blue light on normal tissues? Would the cure be worse than the infection?

I am pointing out these examples, because I hope that they will stimulate people to write better papers, and do a better job of reviewing and editing.

To continue the lecture on radiation biology; radiation survival curves can take many shapes, and analyzing these curves can provide important information **(Figure 3)**. The curve on the right is typical of a bacterial strain that is resistant to radiation. It takes a lot of radiation before the capacity of the cell to cope with the damage is exhausted, and a one-hit line is achieved. The curve on the left is typical of a radiation sensitive mutant of the resistant strain, i.e., one that has lost its capacity to repair its DNA. It shows no shoulder on the survival curve.

Radiation biologists also quantitate information derived from these survival curves; the slope of the lines (D_0), where extensions of the curve crosses the 100% survival line (Dq), or crosses the zero dose line (n), etc. So the conclusion is, if you are going to run radiation biology experiments, you should first learn how to perform radiation biology experiments, and how to plot, and interpret the data.

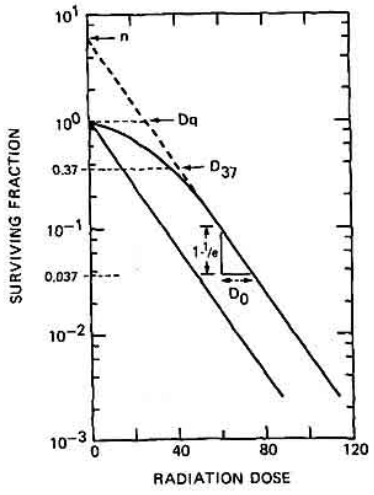

Figure 3. Ultraviolet radiation survival curve for different strains of *Escherichia coli.*

Clearly, the absence of proper scientific training is a big factor in the production of bad papers on LLLT. I don't want to put ALL of the blame on authors for bad papers, I also have to include bad reviewers and bad editors, and, unfortunately, there are a lot of these.

Fortunately, there are only a few basic laws of photobiology, but if researchers and clinicians do not know them, then their studies will be worthless, and they will mislead other people who are similarly untrained. Again, I have to put some of the blame on bad reviewers and bad editors.

The **First Law of Photochemistry** states that: light must be absorbed for photochemistry to occur. This is a very simple concept, but it is the basis for performing photobiological experiments correctly.

Since photobiological and phototherapeutic effects are initiated by photochemistry, unless light of a particular wavelength is absorbed by a system, no photochemistry will occur, and no photobiological effects will be observed, no matter how long one irradiates with that wavelength of light. A significant number of papers in the laser and LED phototherapy literature would not have been published if the authors and the reviewers had known the First Law of Photochemistry.

And now for a word of caution. You should not believe everything that you read. Just because it is published in a book, this does not make it true.

Many of you know that I started the American Society for Photobiology, but I bet that none of you know that I am also considered to be the Father of Photochemistry. It must be true, it is in the book by Tuner and Hode! [2; p.336)]

"According to what is known as 'Kendrick Smith's First Law of Photo Chemistry', light must be absorbed before photochemistry can occur."

Not only do they have it wrong about the First Law of Photochemistry, they also spelled my first name incorrectly. There is no "K" at the end of Kendric. Unfortunately, there are a great number of errors in this book.

The First Law of Photochemistry has been around since the 1800's. Actually, it is known as the Grotthus-Draper Law.

Low Level Light Therapy is real and beneficial, and I get very upset when people publish false statements, or perform bad science and photobiology. Until the LLLT field gets rid of the bad science and falsehoods that are published, LLLT will continue to be considered snake oil medicine by most qualified scientists and physicians.

There are two other laws of photochemistry that should be mentioned for the sake of completeness, even though they have little relevance to phototherapy.

The **Second Law of Photochemistry** states that for each photon of light absorbed by a chemical system, only one molecule is activated for a photochemical reaction.

This law is true for ordinary light intensities, however, with high-powered lasers, two-photon reactions can occur. Two-photon reactions are not important for LLLT.

The **Bunsen-Roscoe Law of Reciprocity:** a photochemical effect is directly proportional to the total energy dose, irrespective of the time required to deliver the dose.

This law is true for chemicals in a test tube, but the response of cells to radiation usually involves a sequence of interacting biological reactions, making a linear "dose x time" relationship highly unlikely. For example, giving a total UV radiation dose to cells all at once kills more cells than the same dose when fractionated. DNA repair systems are induced by the first irradiation, and have a chance to repair much of the damage before the next irradiation.

There is no reciprocity when damage is produced and repaired, but there is reciprocity over a narrow range of doses for photoreceptors that trigger a response, such as phytochrome in plants. The relevancy of reciprocity to LLLT remains to be demonstrated.

An **Absorption Spectrum** is a plot of the probability that light of a given wavelength will be absorbed by the system under investigation. Because of its unique electronic structure, each chemical compound has a different absorption spectrum.

Each of the wavelengths absorbed by a chemical compound will be absorbed to different degrees, again because of the unique electronic structure of the compound. Therefore, an absorption spectrum of the compound that one is interested in will determine the optimum wavelength to use to obtain the maximum absorption with the least amount of light **(Figure 4)**.

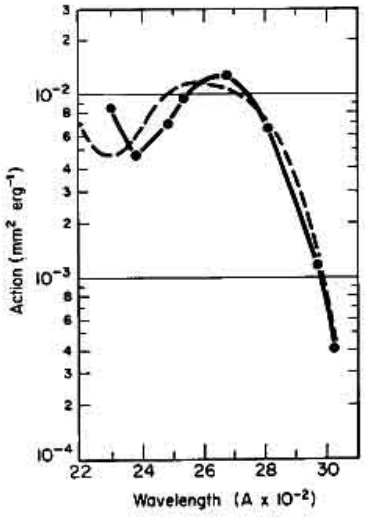

Figure 4. Action spectrum for the killing of *Escherishia coli* by ultraviolet radiation (solid line), and the absorption spectrum for deoxyribonucleic acid (broken line) (modified from 6).

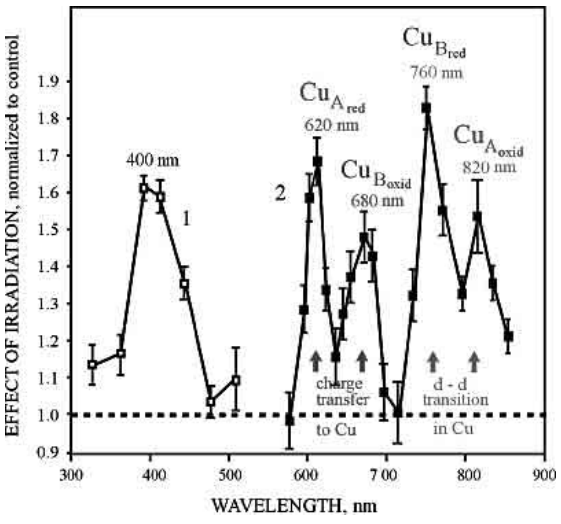

Figure 5. A generalized action spectrum (a summation of 5 action spectra) for the increased proliferation of HeLa cells for wavelengths 330 - 860 nm. [7)]

Of course, not all of the light energy that is absorbed will produce a chemical effect. Some of the absorbed energy can be given off as heat as the electrons move to lower energy levels, and finally, the energy can be emitted as lower energy light, i.e., fluorescence or phosphorescence, to allow the electrons to return to their ground state.

Once a photobiological response is observed, the next step should be to determine the optimum wavelength and dose of radiation to produce the effect, i.e., an action spectrum.

An **Action Spectrum** is a plot of the relative effectiveness of different wavelengths of light in causing a particular biological response. Under ideal conditions it should mimic the absorption spectrum of the molecule that is absorbing the light, and whose photochemical alteration causes the biological effect.

Thus, an action spectrum not only identifies the wavelength(s) that will have the maximum effect with the least dose of radiation, it also helps to identify the target of the radiation. For example, the action spectrum for killing bacteria mimics the absorption spectrum of deoxyribonucleic acid (DNA) **(Figure 4)**. This result is understandable in view of the unique importance of DNA to a cell. This result tells us that if you want to inactivate DNA with the greatest efficiency, i.e., with the least dose of radiation, you should use the wavelength of light at the peak of the absorption and action spectra.

Think of the different wavelengths of light as different drugs. Therefore, it is important to establish which drug is best, and also the optimum dose.

Dr. Tiina Karu has provided us with action spectra for a number of biological end points for cells grown in culture, such as the stimulation of growth, and of DNA and RNA synthesis. Based upon these action spectra, several wavelengths are suggested to be optimal for LLLT, i.e., those around 400, 620, 680, 760, and 820 nm **(Figure 5)**.

This action spectrum is not as simple to interpret as the one shown for the killing of bacteria. However, some of these peaks are identifiable with cytochrome C oxidase, which resides in the mitochondria.

You will note, however, that there are a lot of valleys where the wavelengths are not very active per unit dose. Using a wavelength in one of the valleys might produce the effect that you want, but the dose necessary to produce the effect might be so high that unwanted side effects would probably also be produced.

You can find a review by Dr. Karu on action spectra on the web site, Photobiological Sciences Online, an online textbook on photobiology. [7] There are other papers there on Low Level Light Therapy, as well as papers on all other areas of photobiology.

Determining an action spectrum is a very difficult endeavor, but I wish that someone would do action spectra for some of the clinical effects that are being studied now with random protocols. Then we would finally have a scientific based protocol for treating patients, and the reputation of LLLT would soar.

It must be remembered that most action spectra have been performed on a thin layer of cells. It is possible that the optimum wavelength under these conditions might not be the optimum wavelength for deep tissues. A longer wavelength that penetrates more deeply into tissues might produce the best effect in deep tissues, even though that wavelength was not the most efficient in a thin layer of cells.

To quote from a review by Calderhead: [8] "To sum up, the wavelength of a therapeutic source therefore has a double importance, namely to ensure absorption of the incident photons in the target chromophores, and to be able to do so at the depths at which these chromophores exist. The waveband in which the wavelength of the incident photons is located determines not only which part of the cell is the target, but also the primary photoaction. Wavelength is thus probably the single most important consideration in phototherapy, because without absorption, there can be no reaction."

Another point discussed in this review is that two different wavelengths might produce a better effect under certain conditions, such as wound healing. Calderhead [8] reports that mast cells, neutrophils, and macrophages are the first cells to respond to a wound, and that these cells respond best to 830 nm light. In contrast, fibroblasts, which are involved later, respond better to 633 nm light. The suggestion has been made that it might be better to irradiate first with 830 nm light, followed by 633 nm light, and then again with 833 nm light to activate the myofibroblasts.

The multiplicity of cell types in tissues, and the concept that they may respond better to different wavelengths, does complicate the phototherapy of tissues. It is just one more thing to keep in mind when planning experiments or clinical trials.
A requirement for a good paper on photobiology is to specify everything about the light source, i.e., wavelength(s), power, dose, area of exposure, time, etc., etc.

There are published experimental and clinical studies that were conducted with good scientific methodology, but they did not describe the characteristics of the light source, or they did not even mention the light source. Therefore, these studies cannot be repeated or extended by another author. Such a paper is totally useless. This is like describing a new cure for cancer, without mentioning the name of the drug that was used in the clinical study.

So many acronyms are used in the Low Level Laser Therapy field that it is confusing to readers, e.g., low level laser therapy (LLLT), low-power laser irradiation (LPLI), low power laser therapy (LPLT), low-energy laser irradiation (LELI), etc., etc. It would be a great boon to the field if there could be some standardization of nomenclature. Since lasers just produce light, I would urge the use of the simple and preferred term, PHOTOTHERAPY.

The wavelength of light produced by the laser must be specified, preferably throughout the text of an article in place of acronyms like He-Ne laser.

Also, a laser should be chosen for the wavelength of light that it produces, not because, and I quote from a published paper, "The selection of such a laser for therapeutic use was based on its safety and commercial availability." Whatever happened to WAVELENGTH? This is another example of bad science and bad reviewers.

Even with the proper wavelength and dose of radiation, phototherapy will not be effective on every system and/or situation. The magnitude of the phototherapy effect depends on the physiological state of the cells at the moment of irradiation. For example, when irradiating fresh wounds, the effect of the irradiation can be minimal or nonexistent. This happens when cellular proliferation is active, and the regeneration of the tissue is occurring at a more or less normal rate.

Light will only stimulate cell proliferation if the cells are growing poorly at the time of the irradiation. If a cell is fully functional, there is nothing for radiation to stimulate, and no therapeutic benefit will be observed. An analogy would be that patients will show NO beneficial effect of vitamin therapy if they already receive an adequate supply of vitamins in their daily diet.

It should be cautioned, however, that an excessive dose of radiation can be detrimental. Thus, at proper doses of light there can be a stimulation of growth, but at high doses an excessive amount of singlet oxygen may be produced, and its chemical action can be detrimental to cells. This is another reason for determining an action spectrum.

More and more papers are appearing in the light therapy literature using non-laser light sources, such as LEDs. As with laser studies, all the characteristics of the light emitted by the light source must be specified if a paper is to be useful.

Conclusion

Phototherapy, whether using low intensity radiation of the proper wavelength from a laser, an LED, or a filtered incandescent lamp, can be beneficial in a number of clinical situations, from pain remission to wound healing. Unfortunately, the absence of this type of phototherapy from the mainstream of medicine makes it currently unavailable to many patients who would benefit from it.

The absence of this type of therapy from the mainstream of science and medicine is because so many of the studies have been conducted without proper scientific methodology, and performed by people who lack an understanding of the properties and biological effects of light.

This paper is a plea to scientists, physicians, phototherapy groups, societies and journals, to raise the standards for running and publishing experiments and clinical trials, by learning the basics of photobiology, and thereby accelerating the acceptance of Low Level Light Therapy into the mainstream of science and medicine.

Great progress has been made in the last 10 years, but we still have a way to go.

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