Laser Applications in Biomedicine

Part I: Biophysics, Cell Biology, and Biostimulation

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Abstract

THE SUCCESSFUL applications of lasers to biomedicine rely upon an adequate understanding of the principles of light interaction with tissue. These principles are based upon the fundamentals of photophysics and involve a variety of mechanisms of energy conversion: heat, photochemistry, non-thermal bond breaking, fluorescence, and mechanical shock waves. All of these mechanisms are discussed in the context of biomedical and basic cellular studies. In addition, the mechanism and use of low power (milliwatt) lasers are examined with respect to non-thermal biostimulation phenomena. Many of these applications, such as cell growth stimulation, immunologic response, and wound healing, may be based upon photochemical conversion of absorbed energy.

Introduction and Mechanisms

The purpose of this review is to place the laser in proper perspective; that is to demonstrate where the laser has had major and minor impact, as well as those areas where the laser is controversial and areas where the laser shows potential for future applications.

Since laser light is electromagnetic radiation, the basic principles of photophysics can be applied to understanding the fundamental mechanisms of its interaction with matter [see an excellent review by Boulnois[1]]. It is important to understand these principles because the appropriate and effective use of the laser can best be attained only with an adequate appreciation of them. These mechanisms are simplistically presented in Figure 1. All (except the scattering of light) involve the conversion of the photon energy to some other form of energy that ultimately produces an "effect."

In the applied medical area, the most frequently used mechanism of energy interaction is heat. The first medical use of the laser in the eye utilized the red ruby laser to produce thermal "welds" in the retina. Today, a large number of medical procedures in the eye as well as other areas utilize the argon, dye and krypton lasers to produce selective thermal lesions. In addition, the CO_2 and continuous wave (CW) Nd:YAG lasers are used as thermal devices to coagulate and vaporize tissue in a large number of medical procedures. The most effective use of the laser as a thermal device for tissue alteration necessitates a careful study of the parameters surrounding the application of the radiation: such as power and energy density, wavelength matching with absorption properties of the tissue, thermal conduction properties of the tissue, and thermal time constants. With appropriate attention to these parameters, it is often possible to produce controlled vaporization, cutting, coagulation, or selective tissue "welding." It is even possible to carry the principle of selective "photothermolysis" down to the subcellular level either in tissue[2] or individual cells.[3]

Photon energy may also be dissipated by photochemistry. In this process the absorbing molecule transfers the energy to another molecule which becomes raised to a higher energy state facilitating its interaction with other structures. The most common clinical use of this mechanism has been the excitation of hematoporphyrins which are selectively retained by tumor tissue.[4] The porphyrin molecule absorbs the red photons and transfers the energy to molecular oxygen (which becomes highly reactive singlet oxygen) which reacts with the tumor tissue causing its destruction. In this mechanism, the laser photon energy is passed along from molecule to molecule and ultimately causes its biological effect by the creation of a highly reactive (destructive) chemical species. No heat is generated.

Perhaps the most sophisticated photochemical application of lasers is in biochemical spectroscopy. A very short laser pulse of just a few nanoseconds, picoseconds, or femtoseconds is used to excite molecules into intermediate states that have life times of picoseconds or nanoseconds. Thus, it is possible to study very short lived intermediates in processes such as vision and photosynthesis. This is possible because of the capability to produce extremely short, intense monochromatic emissions with the laser. The mechanism of energy transduction is non-thermal, and the purpose is analytical.

Another process of energy dissipation is the re-emission of energy in the form of light; a process called fluorescence. In this process, the laser provides a monochromatic source of intense light. This mechanism of energy transfer can be used to detect fluorescent species thus identifying binding sights, such as in the detection of cancer tissue by hematoporphyrin fluorescence.[5] Laser-stimulated fluorescence can also be used analytically to scan large populations of cells that are flowed through a laser beam[6] as well as to excite small microscopic regions of single cells to study the binding and movement of biologically important molecules.[7]

With the advent of the short-pulsed, mode-locked (picosecond) and Q-switched (nanosecond) lasers, it became possible to produce very high power densities (gigawatts/cm^2) in focal spots with diameters of 25-50 micrometers. When these beams are focused either in air or tissue, it is possible to produce a plasma which generates a mechanical shock wave. The plasma-generated shock wave from a focused Nd:YAG laser is used routinely to remove secondary cataracts from the posterior capsule membrane in human eyes. In addition, the plasma-gen-

erated shock wave from a pulsed dye laser is being used experimentally to fracture urinary tract stones in human patients. The mechanism of laser energy dissipation and consequent tissue destruction is by a non-thermal physical process.

The most recent application of non-thermal laser disruption of tissue is called "photoablation by molecular bond-breaking." This process was first proposed in the application of 193 nm ArF excimer laser ablation of polymer plastics in the thin silicon film business.[8,9] It was suggested that the combination of high absorption and high individual photon energy resulted in the direct transfer of energy within the absorbing molecule to the bonds that hold the molecule together, thus resulting in a breakdown of the molecule by bond rupture. This was proposed to be a non-thermal process. Though this has not been proven conclusively, structural studies of the affected tissue demonstrate little if any heat damage.[9]

Another process that should be mentioned is ionization. Though it is generally felt that individual photons generated from existing lasers do not have enough energy to cause an ionization process (ejection of electrons from the absorbing molecules), it is possible to have absorption of more than one photon in a "multiphoton process." This has been observed in solutions and in living cells.[10] Such a process could result in enough energy being absorbed to cause ionization.

A final process that should be mentioned is the phenomenon of "optical trapping" by transfer of momentum from photons to a refracting object. Recently described by Ashkin et al.,[11] optical traps can be created that generate enough force to move objects as large as groups of cells or as small as a subcellular structure. This non-destructive use of electromagnetic radiation may become very useful in the study of dynamic biologic processes, such as cell and organelle movement.

In summary, all of the mechanisms discussed have biomedical applications. The most effective use of laser light requires an understanding of these basic photophysical processes.

Cell Biology and Biochemistry

The laser has become a very useful tool in a wide range of studies dealing with basic cellular and biochemical processes. These studies have utilized a variety of the physical characteristics of laser light: (a) monochromaticity, (b) intensity, (c) capability of extremely short pulse durations (nanosecond, picosecond and femtosecond), and (d) Gaussian geometry of beam profiles.

One of the earliest laser applications was in the area of cellular microsurgery, where the laser was focused through a microscope to a submicron spot diameter to produce a subcellular lesion inside of a single living cell.[3,12,13,14] The spatial resolution of this technique was dependent upon the fact that the laser beam had a Gaussian energy distribution which resulted in a threshold lesion size in the focused spot equivalent to the width of the central peak of the Gaussian. Thus it was possible to produce subcellular lesions as small as 0.25 microns in diameter. This technique has been used to study the struc-

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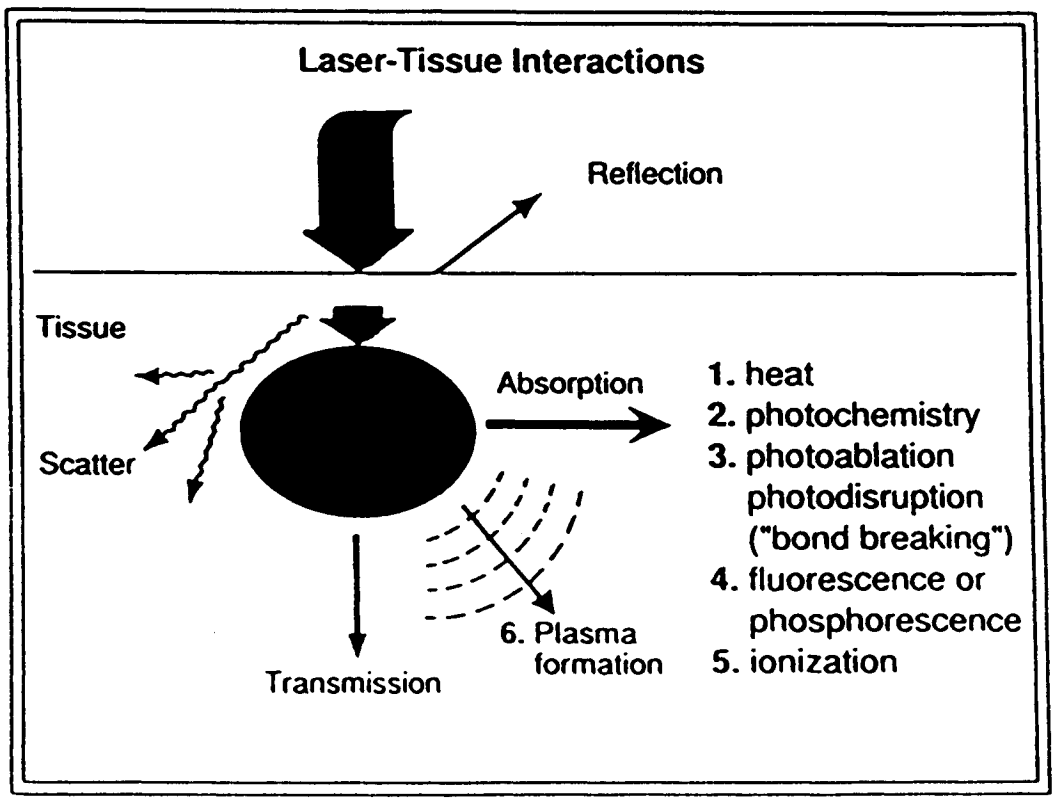

FIGURE 1: Laser tissue interactions

ture and functions of numerous cell structures: chromosomes, mitochrondria, nucleoli, cell membrane, nuclear envelope, and mitotic structures.[3] More recently, this technique has been used to perforate the cell membrane in order to facilitate the uptake and stable incorporation of foreign DNA into animal cells in culture.[15,16] The ultimate goal of these genetic experiments is to apply this technology to the field of plant genetic engineering where it is technically very difficult to introduce DNA into many economically important plants.

In addition to using a microscopically focused laser to ablate specific subcellular structures, the laser can be focused through the microscope for fluorescence analysis. This can be used for subcellular localization of specific molecules, such as mitochondria-bound Rhodamine 6G[17] or membrane-bound dyes that fluoresce at different wavelengths when the membranes are in different physiological states. The most widely applied laser-induced fluorescence is in the area of fluorescence recovery after photobleaching (FRAP) studies.[7] In these studies, the laser is focused onto either the outer cell membrane or some internal cell region where a fluorescent probe has already been bound. A baseline fluorescent reading is taken with the laser at a very low output sufficient only to excite fluorescence. Then an intense pulse of laser light is delivered to the same subcellular spot causing a photodegradation of the fluorescent molecules. Next the laser is allowed to remain on at a low intensity in the same focused spot so that the time course of fluorescence recovery in that spot can be monitored. This measurement, termed FRAP, provides a means to measure the movement of unbleached fluorescent molecules back into the exposed spot, thus providing a real-time measurement of the mobility of the molecules in the cell structure being studied. In the case of the cell membrane, FRAP measurements have provided very useful information on the physiology and structure of the cell membrane as well as on the binding and mobility of cell membrane receptors in different cell types and at different stages of development.[18]

Laser-induced fluorescence and light scattering has been used for years in a non-microscopic mode to provide quantitative information about a large number of cells using systems called flow cytometers. This approach involves the rapid flow of a stream of cells in solution through a laser beam (usually an argon laser). If the cells have been pretreated with a suitable fluorescent probe (such as DNA-binding), the fluorescent signal given off as the cell passes through the laser beam can be used to measure some characteristic of the cell (such as the amount of DNA in the cell). In this way, thousands of cells can be measured in just a few minutes and the information can be collated and tabulated by computer techniques.[6] This technique can also be used to separate out populations of cells with desired characteristics through sophisticated computer-based methods in a system that is called a cell sorter. In addition to measurements being made based upon fluorescence, flow cytometers can also make measurements based upon the scattering of the laser light by the cells as they pass through the beam. This permits measurement and sorting based upon cell size without having to expose the cells to a foreign fluorescent probe. These systems are routinely used in both research and diagnostically in clinical labs to measure blood cell types.

In the area of biochemistry, the laser is used quite extensively as a spectroscopic tool to investigate biological reactions that occur on a very fast time scale. In these studies, a very short pulse of laser light, on the order of a few picoseconds (10^{-12}s) or femtoseconds (10^{-15}s), is delivered to the biological system, and the chemical species that are produced are studied spectroscopically utilizing very fast detectors (streak cameras). The laser is needed because it is the only light source that can produce sufficiently intense, monochromatic light in such a short time scale. Since the chemical species produced may only have a lifetime of a few picoseconds or nanoseconds, the exciting light must be of shorter duration. Spectroscopy in the picosecond and nanosecond time domains has permitted study of the most fundamental aspects of energy transduction in vision,[19] and photosynthesis.[20]

Low Power Laser Effects: Biostimulation

Laser "biostimulation" refers to a group of biological phenomena that are generated as a result of photons from a low power laser (milliwatts/cm^2) that does not appear to involve a temperature rise as the main mechanism of energy transfer. The types of "biostimulatory" phenomena that have been described can be categorized into several groups:

1. *in vitro* cellular effects on growth, biochemistry and motility;
2. wound healing *in vivo* (ulcers and skin lesions);
3. immunological activation/suppression *in vivo* and *in vitro*;
4. neurophysiological responses *in vitro* (cellular) and *in vivo* (pain control and acupuncture).

For years, Mester et al.[21] have been describing a variety of cellular and *in vivo* systems where a "laser biostimulatory" phenomenon was implicated. For example, white blood cells exposed to 0.05 J/cm^2 of ruby laser radiation at 694 nm increased their phagocytotic activity of bacteria. However, they also found an inhibitory effect of the same radiation at higher energy densities (2-4 J/cm^2). In another *in vitro* study, the same authors[22] exposed Ehrlich ascites tumor cells to low power radiation from the ruby laser and then injected the irradiated cells into mice where the tumor cells proliferated at a greater rate than unirradiated injected cells. This result suggested a growth stimulatory effect of the low power red ruby laser.

Similarly, studies involving the direct exposure of bacterial cells in culture to 1 J/cm^2 of red ruby laser energy (694 nm) caused a pronounced increase in growth rate, but a higher laser dose of 120 J/cm^2 produced an inhibitory phenomenon reminiscent of the study on cellular phagocytosis. The biostimulation of protein synthesis was detected in a study where hemoglobin synthesis was observed to increase following 0.5-20 J/cm^2 of ruby laser exposure.[23]

In another study, the same group examined the effects of the helium neon (624 nm), the argon laser (488 and 514 nm), and the ruby laser (694 nm) on the growth of T and B immunologic cells *in vitro*. In this study, they suggested

that the growth effects were highly dependent on the polarization of the laser beam.

In a more recent study, Abergel et al.[24] undertook to determine if collagen synthesis *in vitro* could be affected with a YAG laser (1.06 um) by a non-thermal mechanism. In this study, the YAG laser was focused to a .15 cm^2 spot into tissue culture wells containing human skin fibroblasts that were capable of synthesizing collagen *in vitro*. The laser power density was 390 mW/cm^2 (a level that could cause a temperature rise) and total laser energy was 1-5 x 10^3 J/cm^2. The authors examined collagen synthesis, DNA, RNA, and protein synthesis in the laser-treated cells, and in control cells that were heated to similar temperatures. Only the laser-treated cells exhibited a decrease in all three synthetic processes.

The most extensive series of studies to date on cell systems are those of Karu and colleagues in the U.S.S.R.[25]. In these studies, action spectra were carried out on yeast cells, bacteria and eukaryotic HeLa cells in culture. The effects of different wavelengths were examined on parameters, such as cell growth rate, DNA and RNA synthesis, and a variety of respiratory enzyme systems. Using relatively low intensities (10^{-2}-10^{-4} W/cm^2) and total fluences in the range of 10^2-10^4 J/cm^2, wavelength dependencies were observed. In fact, absorption differences between the respiratory chain molecules of the different organisms were reflected in the action spectrum analyses. These results strongly suggest that some of the cellular based "biostimulatory" phenomena are probably due to absorption (and subsequent energy conversion) by respiratory chain molecules.[25]

Wound Healing

Mester et al.[26] initially studied wound healing in a mouse skin model system. In those studies, open skin wounds were exposed to red ruby laser radiation (649.3 nm) twice weekly to a total dose of 1.1 J/cm^2. Under these conditions, the investigators found an increase in cell proliferation around the lips of the wounds, an increase in tissue granulation (a sign of wound healing) and a quicker rate of wound closure than normal.

Studies using radioactive precursors for proteins suggested that the main effect of the laser exposure was on the synthesis of collagen.[27] It was suggested that the nuclear-containing vesicles that were found in the light-exposed tissue might have been indicative of some "bioactive" substance that was produced.

The same authors[28] followed up on the "bioactive" substance idea by investigating the stimulatory effect of the laser on leukocyte phagocytotic activity. They found that cell suspension irradiation followed by collection of the "supernatant" from the cells and subsequent exposure of other leuckocytes of this supernatant, resulted in a stimulation of phagocytosis in these cells. This suggested that a "bioactive" substance had been released from the irradiated cells.

Another experiment by the same group involved the examination of enzyme activity by cytochemical staining following exposure to the low energy laser. It was found that within a 8-48 hour period of laser exposure to wounds, there was an increase above control levels of SDH, LDH, and acid phosphatase in the basal epithelial cells of the wound.

Despite the consistent demonstration of a wound healing effect of low level laser radiation by the Eastern European research group, several attempts to repeat these phenomena by others have been unsuccessful. In a study by Hunter et al.,[29] low energy helium neon laser energy was used to expose skin wounds in the pig. These authors found no difference in healing between exposed and unexposed lesions.

In another study, Surinchak et al.[30] looked at helium neon laser stimulation of skin wounds in rats and rabbits. These studies involved the use of a 50 milliwatt HeNe laser with total light doses of 1-5 J/cm^2. The authors found a 55% increase in tensile strength in the laser-treated animals within a 14 day post-treatment period, but by 28 days tensile strength was the same for both laser treated and non-treated controls. This study did suggest some types of early laser-stimulation effects, but from a clinical point of view, no long-term useful effect was observed.

Another study has been conducted by Jongsma et al.[31] using an argon laser at 488 and 514 nm comparing wavelength, energy density, and power density on the wound healing in the rat. They compared their results with the results of others who had used the HeNe and ruby laser. These authors found no wound healing effect, but they do suggest a wavelength dependency when their results are compared to those of others.

Immunological and Anti-Inflammatory Action

A direct stimulatory effect of the laser on the immune system has been suggested. In an early study,[32] it was demonstrated that the survival rates of laser-treated skin transplanted to "foreign" mice survived with 28% better efficiency than non-laser treated skin. In these studies, the skin was exposed to the unfocused HeNe laser at 20 mW prior to being transplanted.

In another study by the same group,[33] lymphocyte transformation was shown to increase when the laser was used in combination with phytohemagluttin (PHA), a substance that promotes the transformation. The laser used in these studies was the red ruby laser at 1 J/cm^2.

In addition to the previous two studies, a direct effect of the HeNe, ruby and argon lasers on the T and B cells of the immune system has been described. In this study, it was demonstrated that optimal immunosuppressive effects occurred when CW and pulsed laser sources were combined.

An earlier study by another group[34] demonstrated that unfocused ruby laser irradiation of the spleen in white rats treated with *Brucella abortus* had a decreased immunological reaction to the infection. This effect was attributed to both a decrease in the number of immune cells produced in response to foreign protein challenge and a decrease in the titer to *Brucella* antibodies produced by the irradiated host animal.

A study by Moskalik et al.[35] appeared to demonstrate an increase in spleen antibody-forming cells 7-14 days fol-

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lowing neodymium glass laser exposure of experimental tumors growing on the hind limbs of rats. However, with time, these and other immuno-stimulated responses returned to normal.

Most recently, Ohta et al.[36] have demonstrated an inhibition of lymphocyte proliferation *in vitro* following exposure to 1-85 mJ/cm² of a galium arsenide laser operating at 904 nm.

The possible anti-inflammatory action of the laser is worthy of mention at this point since it may relate to an immunological effect of the laser. In one of the early studies, it was suggested that the HeNe laser operating at 50 mW with a 1 J/cm² energy density presumably caused an increase in prostaglandin release following irradiation of skin. It was suggested that the effect was primarily on the inflammatory phase of the wound healing process. This effect may be extrapolated to the two studies published by Goldman[37,38] that appear to show a direct effect of low level laser exposure on the rheumatoid arthritic joints of patients. The first study[37] appeared to document improvement of the joints, and the second study[38] compared irradiated with unirradiated joints. The laser used was the Nd:YAG laser operating at 1.06 um in the Q-switched modes. The power densities were relatively high, but the total energy density was low. The results of the latter study appeared to demonstrate improvement in both laser treated and untreated joints in the same patient. However, the improvement in the laser treated joints was much greater than in the untreated joints. Parameters examined were erythema, pain, grasp strength, tip pinch, and flexion. Systemically, immune complexes decreased following the laser exposure. This study appears to support the studies of other investigators suggesting an immunosuppressive effect of the laser, perhaps through the release of some "bioactive" substances from the irradiated site. However, no definitive experimental investigations to confirm these observations have been conducted.

Neurological/Neurophysiological Responses

A study by Fork[39] was the first to suggest a direct effect of the laser on nerves. The laser used was an argon ion CW emitting 4.5-12.5 mW at 488 nm. Since the laser was focused to a 10 um diameter spot, the power density was in the range of 75-200 mW/cm². The exposure durations were 2-5 sec thus keeping the total energy to less than 1 J/cm². The net result of these studies was the demonstration of an induction of membrane depolarization by the laser exposure. In another study involving irradiation of intestinal mucosa with 1-3 J/cm² of the ruby laser,[40] an increased motility of the intestinal mucosa was observed. It was suggested by the authors that the result was due to a direct effect on the nerves in the intestine. A study conducted by Vizi et al.[41] appeared to demonstrate that a ruby laser (694 nm at 9 J and 0.5 ms duration, pulse rate of 9.5 Hz for 10 min) was able to stimulate the release of acetylcholine (ACH) from the Auerbach's Plexus in the guinea pig ileum. The authors theorized that the ACH stimulation was a result of a direct non-thermal effect of the laser on the neural plexus.

Even though there has not been extensive *in vitro* or *in vivo* animal work with the low energy laser in neurological systems, two rather extensive human clinical applications appear to be evolving. There is a vast body of published observations in the Chinese literature dealing with the analgesic effect of the low power HeNe (1-10 mW) when applied to acupuncture points. Unfortunately, most of these studies have not been translated into English; therefore, it is often difficult to assess the results. Notwithstanding, Yo-Cheng[42] has published a study in English describing the use of the HeNe laser to induce an anesthetic response in over 600 patients undergoing dental treatment. The proposed mechanism is some form of "biostimulatory" phenomenon at or near the level of the skin surface. In another clinical series, Walker[43] has described the use of a low power (1 mW) HeNe laser to relieve pain in patients suffering from chronic pain. A double blind study was described where significant pain relief was observed when the skin overlying the radial, medial, and saphenous nerves was irradiated. However, neither of these applications has gained acceptance clinically or scientifically.

Conclusion

A survey of the diffuse literature of low power "laser biostimulation" reveals a somewhat confusing and muddled field. Taken as a whole, the wide variety of biological systems that appear to exhibit some change under the influence of low fluence laser exposure would suggest that there is some form of lower laser power effects that may not be explained by simple temperature effects. Whether or not the mechanism(s) are the same for the different systems (i.e., wound healing vs. pain relief) cannot be stated at this time. The recent studies of Karu[25] and earlier studies of Salet et al.[44,45] strongly implicate a photochemical mechanism via direct absorption by respiratory chain molecules.

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Laser Applications: Part II — Clinical Applications, will appear in a future issue.

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