(PHOTOBIOMODULATION)

1. What is photobiomodulation (low-power laser therapy?)

More than 30 year ago the first publications about low-power laser therapy or photobiomodulation (at that time called laser biostimulation) appeared. Since then approximately 2000 studies have been published on this topic (analysis of these publications can be found in [1]). Medical treatment with coherent light sources (lasers) or noncoherent light (Light Emitting Diodes, LED's) has passed through its childhood and early maturity. Photobiomodulation is being used by physiotherapists (to treat a wide variety of acute and chronic muscosceletal aches and pains), dentists (to treat inflamed oral tissues, and to heal diverse ulcerations), dermatologists (to treat oedema, indolent ulcers, burns, dermatitis), rheumatologists (relief of pain, treatment of chronic inflammations and autoimmune diseases), and by other specialists (e.g., for treatment of middle and inner ear diseases, nerve regeneration). Photobiomodulation is also used in veterinary medicine (especially in racehorse training centers) and in sports medicine and rehabilitation clinics (to reduce swelling and hematoma, relief of pain and improvement of mobility and for treatment of acute soft tissue injuries). Lasers and LED's are applied directly to respective areas (e.g., wounds, sites of injuries) or to various points on the body (acupuncture points, muscle trigger points). For details of clinical applications and techniques used, the books [1-3] are recommended.

2. What light sources (lasers, LED's) can be used?

The field of photobiomodulation is characterized by variety of methodologies and use of various light sources (lasers, LED's) with different parameters (wavelength, output power, continuous wave or pulsed operation modes, pulse parameters). These parameters are usually given in manufacturers manuals.

The GaAlAs diodes are used both in diode lasers and LED's, the difference is whether the device contains the resonator (as the laser does) or not (LED). In latter years, longer wavelengths (-800-900 nm) and higher output powers (to 100 mW) are preferred in therapeutic devices.

Should a medical doctor use a laser or a diode? The answer is - it depends on what one irradiates, in other words, how deep tissue layers must be irradiated. By light interaction with a biotissue, coherent properties of laser light are not manifested at the molecular level. The absorption of low-intensity laser light by biological systems is of a purely noncoherent (i.e., photobiological) nature. On the cellular level, the biological responses are determined by absorption of light with photoacceptor molecules (see the section 3 below). Coherent properties of laser light are not important when cellular monolayers, thin layers of cell suspension as well as thin layers of tissue surface are irradiated (Fig. 1). In these cases, the coherent and noncoherent light (i.e., both lasers and LED's) with the same wavelength, intensity and dose provides the same biological response. Some additional (therapeutical) effects from the coherent and polarized radiation (lasers) can occur in deeper layers of bulk tissue only and they are connected with random interference of light waves. An interested reader is guided to the ref. [4] for more details. Here we illustrate this situation by Fig. 1. Large volumes of tissue can be irradiated by laser sources only because the length of longitudinal coherence Lcoh is too small for noncoherent radiation sources [4].

3. Enhancement of cellular metabolism via activation of respiratory chain: a universal photobiological action mechanism

A photobiological reaction involves the absorption of a specific wavelength of light by the functioning photoacceptor molecule. The photobiological nature of photobiomodulation means that some molecule (photoacceptor) must first absorb the light used for the irradiation. After

promotion of electronically excited states, primary molecular processes from these states can lead to a measurable biological effect **(via secondary biochemical reaction, or photosignal transduction cascade, or cellular signaling)** at the cellular level. The question is, which molecule is the photoacceptor.

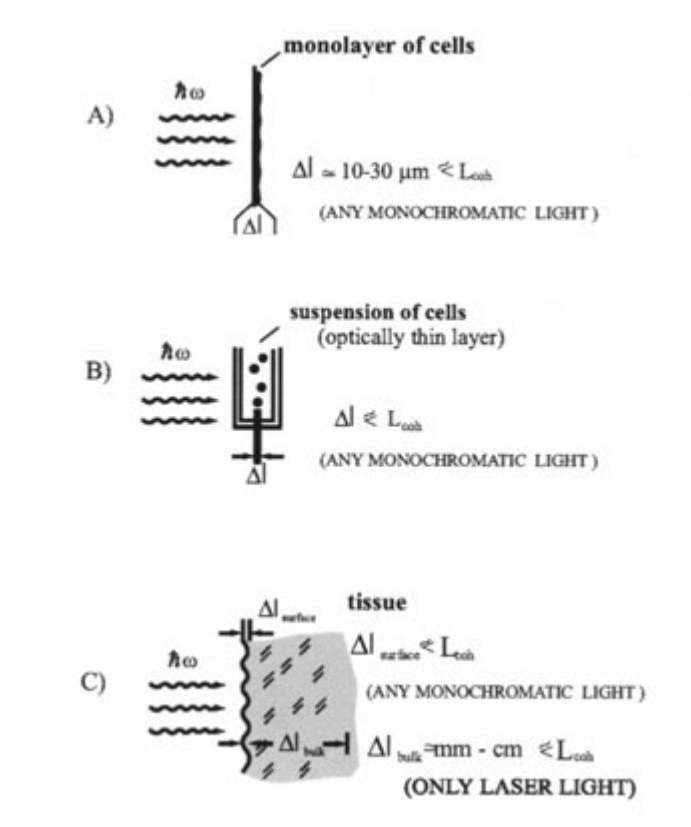

Fig. 1. Depth (On in which the beam coherency is manifested, and coherence length Lcoh in various irradiated systems: (A) monolayer of cells, (B) optically thin suspension of cells, (C) surface layer of tissue and bulk tissue. Lcoh, - length of temporal (longitudinal) coherence of laser light, hw) marks the radiation.

When considering the cellular effects, this question can be answered by **action spectra**. Any graph representing a photoresponse as a function of wavelength, wave number, frequency, or photon energy, is called action spectrum. Action spectra have a highest importance for identifying the photoacceptor inasmuch as the action spectrum of a biological response resembles the absorption spectrum of the photoacceptor molecule. Existence of a structured action spectrum is strong evidence that the phenomenon under study is a photobiological one (i.e., primary photoacceptors and cellular signaling pathways exist). Fig. 2 represents some examples of action spectra for eukaryotic cells: two of them (A, B) consider the processes occurring in cell nucleus, and one spectrum (C) is for cell membrane. Fig. 2D shows the absorption spectrum of the monolayer of the same cells.

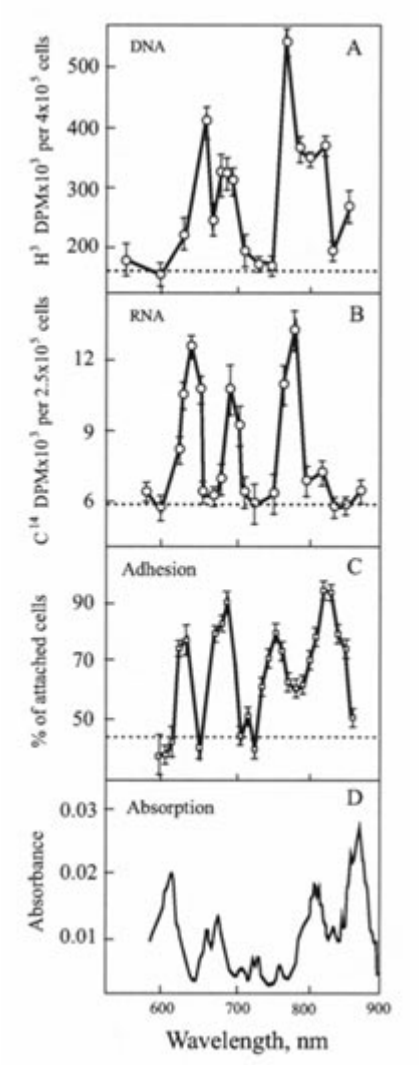

Fig. 2. Action spectra of: (A) DNA and (B) RNA synthesis rate in HeLa cells; (C) plasma membrane adhesion of HeLa cells for red-to-near IR radiation; (D) absorption spectrum of air-dried monolayer of HeLa cells for the same spectral region. Original data can be found in ref. [5].

The spectra in Fig. 2 represent the red-to-near infrared (IR) region only, i.e. the region that is most important for photobiomodulation. The action spectra for full visibleto-near IR region can be found in [5]. In [5] one can find action spectra for various cellular responses for other eukaryotic and prokaryotic cells as well.

Two conclusions can be drawn from action spectra in Fig. 2. First, the similarity of the action spectra for different cellular responses suggests that the primary photoacceptor is the same for all these responses. Second, the existence of the action spectra for biochemical processes occurring in various cellular organelles (nucleus, Fig. 2A, B and plasma membrane, Fig. 2C) assume the existence of cellular signaling pathways inside of a cell between the photoacceptor and the nucleus as well as between the photoacceptor and cell membrane. Action spectra also indicate, which wavelengths are the best for irradiation: maximal biological responses are occurring when irradiated at 620, 680, 760 and 820-830 nm (maxima of the spectra in Fig. 2). Skipping over the story of identifying the photoacceptor (described in [5]) let us conclude that

photoacceptor for eukaryotic cells in red-to-near IR region is believed to be the terminal enzyme of the respiratory chain **cytochrome c oxidase** (located in cell mitochondrion). To be more exact, it is a mixed valence (partially reduced) form of this enzyme, which has not yet been identified. In the violet-to-blue spectral region, flavoproteins (e.g., NADHdehydrogenase in the beginning of the respiratory chain) are also among the photoacceptors as well terminal oxidases.

An important point has to be emphasized. When the excitable cells (e.g., neurons, cardiomyocites) are irradiated with monochromatic visible light, the photoacceptors are also believed to be components of respiratory chain. Some of the experimental evidence concerning excitable cells is shortly summarized in Fig. 3. It is quite clear from experimental data (reviewed in [4]) that irradiation can cause physiological and morphological changes in nonpigmental excitable cells via absorption in mitochondria. Later, similar irradiation experiments were performed with neurons in connection with low-power laser therapy. It was shown in 80's that He-Ne laser radiation alters the firing pattern of nerves; it was also found that transcutaneous irradiation with HeNe laser mimicked the effect of peripheral stimulation of a behavioral reflex. These findings were found to be connected with pain therapy (review [4])

.

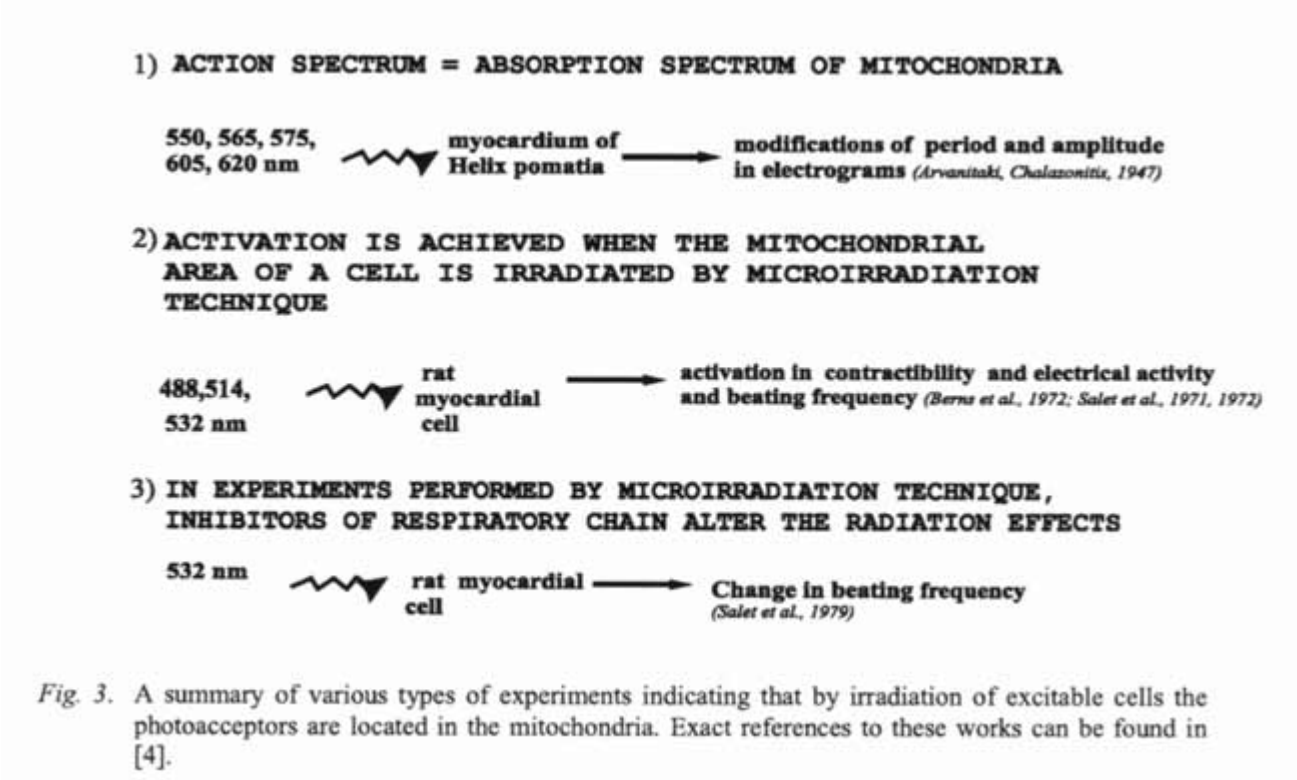

Fig. 3. A summary of various types of experiments indicating that by irradiation of excitable cells the photoacceptors are located in the mitochondria. Exact references to these works can be found in [4].

So, what happens when the molecule of photoacceptor absorbs photons? Answer - electronic excitation followed by photochemical reactions occurring from lower excitation states (first singlet and triplet). It is also known that electronic excitation of absorbing centers alters their redox properties. Until yet, five primary reactions have been discussed in literature (Fig. 4). Two of them are connected with alteration of redox properties and two mechanisms involve generation of reactive oxygen species (ROE). Also, induction of local transient (very short time) heating of absorbing chromophors is possible. Details of these mechanisms can be found in [4, 5].

There is no ground to believe that only one of the reactions shown in Fig. 4 occurs when a cell is irradiated and excited electronic states are promoted. The question is, which mechanism is decisive. It is not excluded that all mechanisms shown in Fig. 4 lead to a similar result, to a modulation of redox state of the mitochondria (a shift to more oxidized direction). However,

depending on the light dose and intensity used, some mechanism(s) can prevail significantly [5].

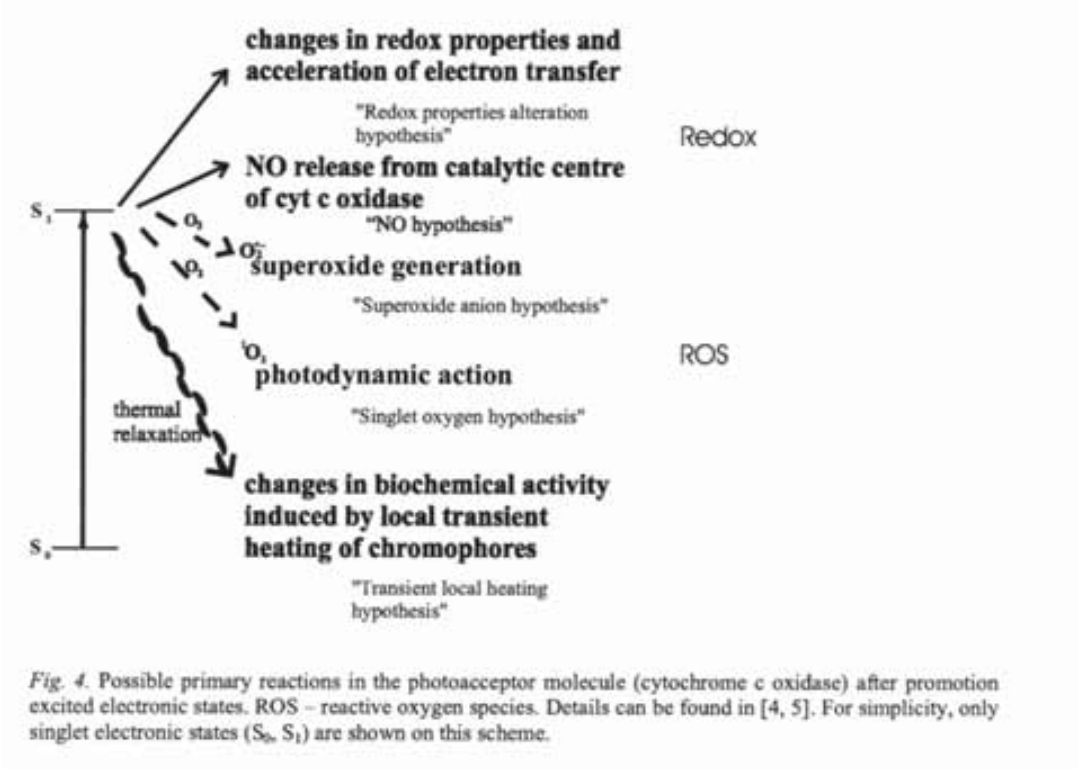

Fig. 4. Possible primary reactions in the photoacceptor molecule (cytochrome c oxidase) after promotion excited electronic states. ROS – reactive oxygen species. Details can be found in [4, 5]. For simplicity, only singlet electronic states (S_0, S_1) are shown on this scheme.

The next question is, the following if photoacceptors are located in the mitochondria, then how the **primary reactions occurring under irradiation** in the respiratory chain (Fig. 4) are connected with DNA and RNA synthesis in the nucleus (the action spectra in Fig. 2A, B) or with changes in plasma membrane (Fig. 2C)? The principal answer is that between these events there **are secondary (dark) reactions (cellular signaling cascades or photosignal transduction and amplification, Fig. 5)**.

Three regulation pathways are suggested in Fig. 4. The first one is the control of photoacceptor over the level of intracellular ATP. It is known tat even small changes in ATP level can alter cellular metabolism significantly. This regulation way is especially important by irradiation of hypoxic, starving or otherways stressed cells. However, in many cases the regulative role of redox homeostasis is proved to be more important than that of ATP. For example, it is known that the susceptibility of cells to hypoxic injury depends more on the capacity of cells to maintain the

redox homeostasis and less on their capacity to maintain the energy status.

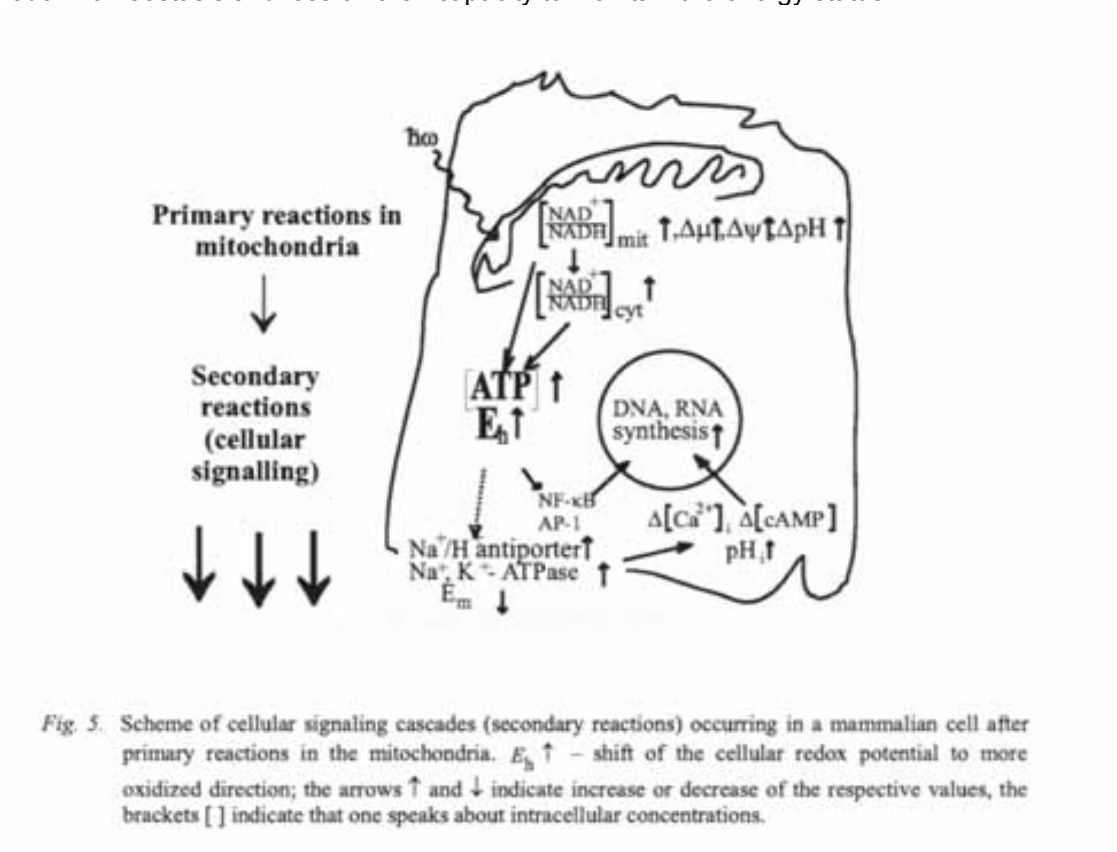

Fig. 5. Scheme of cellular signaling cascades (secondary reactions) occurring in a mammalian cell after primary reactions in the mitochondria. E_h ↑ – shift of the cellular redox potential to more oxidized direction; the arrows ↑ and ↓ indicate increase or decrease of the respective values, the brackets [ ] indicate that one speaks about intracellular concentrations.

The second and third regulation pathways are mediated through the cellular redox state (Eh; Fig. 4). This way involve redox-sensitive transcription factors (NF-KB and AP1, Fig. 4) or cellular signaling homeostatic cascades from cytoplasma via cells membrane to the nucleus (Fig. 4). As a whole, the scheme in Fig. 4 suggests a shift in overcell redox potential into more oxidized direction. Modulation of cellular redox state affects gene expression namely via transcription factors. It is important that in spite of some similar or even identical steps in cellular signaling, the final cellular responses to the irradiation differ due to existence of different modes of regulation of transcription factors. The mechanisms of regulation are not understood well yet.

The magnitude of cellular responses depends on cellular redox potential (and its physiological status, respectively) at the moment of irradiation. The cellular response is stronger when the redox potential of the target cell is initially shifted to a more reduced state (and intracellular pH, pH;, is lowered, as usually happens in injured cells). This explains why the degrees of cellular responses can differ markedly in different experiments or in different clinical cases, and why the effects are sometimes nonexistent.

One should emphasize that some biological limitations exist for photobiomodulation effects. These are discussed in [5].

4. Enhancement of cellular metabolism via activation of nonmitochondrial photoacceptors. Indirect activation/suppression

The redox regulation mechanism cannot occur solely via respiratory chain (Section 3). Other redox chains containing molecules, which absorb light in visible-to-near IR radiation, and are some key structures that can regulate a metabolic pathway, can be photoacceptors for

photobiomodulation as well. One such example is NADPH-oxidase of phagocytic cells, which is responsible for nonmitochondrial respiratory burst. This multicomponent enzyme system located in the plasma membrane is a redox chain that generates reactive oxygen species (ROS) as a response to the microbicidal or other types of activation. Irradiation with He-Ne laser and diode lasers and LED's can activate this chain in various phagocytic cells. Many worked examples can be found in [5]. In phagocytes, the activation of respiratory chains in mitochondria occurs as well, as NADHP-oxidase activation, but the latter is much stronger.

ROS, burst of which is induced by direct irradiation of phagocytes, can activate or inactivate other cells, which were not irradiated directly. In this way, indirect activation or suppression of metabolic pathways in non-irradiated cells occurs. Also, lymphokines and cytokines produced by irradiated lymphocytes can influence metabolism of other cells. This situation is common by irradiation on tissues.

5. Concluding Remarks

The photobiological action mechanism via activation of respiratory chain is a universal working mechanism for various cells. Crucial events of this type of cell metabolism activation are occurring due to a shift of cellular redox potential into more oxidized direction as well as due to ATP extrasynthesis. Susceptibility to irradiation and capability for activation depend on physiological status of irradiated cells: the cells, which overall redox potential is shifted to more reduced state (example: some pathological conditions) are more sensitive to the irradiation. The specificity of final photobiological response is determined not at the level of primary reactions in the respiratory chain but at the transcription level during cellular signaling cascades. In some cells, only partial activation of cell metabolism happens by this mechanism (example: redox priming of lymphocytes).

All light-induced biological effects depend on the parameters of the irradiation (wavelength, dose, intensity, irradiation time, and continuous wave or pulsed mode, pulse parameters). According to action spectra, optimal wavelengths are 820-830, 760, 680, and 620 nn. Large volumes and deeper layers of tissues can successfully irradiated by laser only (e.g. inner and middle ear diseases, injured siatic or optical nerves, deep inflammations etc.). The LED's are excellent for irradiation of surface injuries.

Cited Literature

1. Tuner, J. and Hode, L. (1999). Low Level Laser Therapy. Clinical Practice and Scientific Background. Prima Books, Grangesberg (Sweden).

2. Baxter, G.D. (1994). Therapeutic Lasers. Theory and Practice. Churchill Livingstone, London.

3. Simunovic, Z., editor (2000). Lasers in Medicine and Dentistry, vol. I. Vitgraf, Rijeka (Croatia).

4. Karu, T.I. (2002). Low power laser therapy. In: CRC Biomedical Photonics Handbook, T. Vo-Dinh, Editor- in-Chief, CRC Press, Boca Raton (USA).

5. Karu, T.I. (1998). The Science of Low Power Laser Therapy. Gordon and Breach Sci. Publ., London.