Optical Dosimetry for Controlling the Efficiency of Laser Phototherapy

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***Abstract.* – The results in vivo investigation biophotonics of laser-induced photodissosiation of oxyhemoglobin in cutaneous blood vessels and its role in biomedical processes are presented. New method for determination an individual response to the effect of laser radiation is presented. It is shown that in order to make the phototherapy as well as laser therapy methods really efficient one has to control the oxygen concentration in tissue keeping it at the necessary level. Novel method of optical "dosimetry" based on using the changes oxygen concentration in tissue as feedback signal for optimization therapeutic effect of low intensity laser radiation is developed.**

Keywords – hemoglobin, oxyhemoglobin, tissue oxygenation, hypoxia, phototherapy, photodissociation.

I. INTRODUCTION

Biophotonics of "laser-tissue" interaction and the effect of laser radiation on oxyhemoglobin in cutaneous blood vessels and capillaries is considered as one of the interesting aspects of modern photomedicine and photobiology. Application of low intensity laser radiation in treatment of a variety of diseases has been developed extensively during the last five decades.

Biostimulation and therapeutic effect of laser radiation is well-established fact and currently widely uses in clinical practice. At the same time the mechanism of therapeutic effect of laser radiation is not yet clearly understood and considered to be very complex that involves anti-inflammatory, analgesic and anti-edematous effect on tissue [1-4]. Most exiting effect of laser therapy could be seen in wound healing were process of fast epitelization clearly demonstrate its efficiency.

The mechanism of therapeutic effect of laser radiation is still remains unclear that make difficult to develop correct method for controlling the efficiency of laser therapy - correct "dose" of delivered average energy of laser radiation.

In present the efficiency of therapeutic effect is controlled by using an empirical unit based on average power density of output laser radiation. Experimental study the therapeutic effect of He-Ne and Argon laser radiation in open skin wound healing [5] was carried out at power density of 45mW/cm^2. Maximal therapeutic effect due to significant increase of collagen synthesis at the total energy density of 4J/cm^2 has been reached. Similar experimental study [6] with He-Ne laser radiation at power density of 4,0mW/cm^2 demonstrated the therapeutic dose (complete heeling of wound) at lower average energy ~1,22J/cm^2.

Big differences in experimental results in healing two cases of identical open wounds where therapeutic effect are reached in different output power of He-Ne laser radiation remains not clear.

Nevertheless the power density of 4J/cm^2 is accepted as extreme level ("dose") for reaching maximal therapeutic effect. Accepted empirical criterion for controlling the efficiency of the therapeutic effect of low intensity laser radiation (optical "dosimetry") is not correct and reliable.

In this paper new method of optical "dosimetry" based on using the changes of oxygen concentration as feedback signal for optimization of therapeutic effect of laser radiation is presented. It is shown that photodissociation of oxyhemoglobin; whose main biological function is the transportation of molecular oxygen, gives unique possibility of additional oxygen supply and allows develop laser-optical method of tissue hypoxia elimination for restoring normal cell metabolism.

II. THE PHENOMENON OF LASER-INDUCED BLOOD OXYHRMOGLOBIN PHOTODISSIATION

Since 1997 new technology of laser-induced photodissociation of oxyhemoglobin (HbO_2) in cutaneous blood vessels and its biomedical applications is developing. Unique possibility in selective and local increase of the concentration of free molecular oxygen in tissue is obtained [7-10]. The efficiency of the interaction of laser radiation in different wavelengths on HbO_2 in cutaneous blood vessels is studied. Mathematical model for calculating optimal parameters of laser radiation to induce an effective photodissociation of hemoglobin (Hb) complexes in cutaneous blood vessels has been developed.

The temperature dependence of the quantum yield of photodissociation of HbO_2 observed earlier in vitro is proved experimentally in vivo [10]. Unique possibility in selective and local increase of the concentration of free molecular oxygen (O_2) in tissue is demonstrated.

As it well known the concentration of oxygen is critical in enhancing in vivo wide variety of biochemical reactions including cell metabolism. Aerobic cell metabolism is primary mechanism in energy production in tissue. Controlling this mechanism gives unique possibility of biological stimulation to reach therapeutic effect. This goal could be reached by the means of laser-induced photodissociation of oxyhemoglobin in cutaneous blood vessels.

Absorption of light by blood Hb and HbO_2 allows consider and discuss the following photophysical and photochemical processes. Photophysical process is connected with nonradiative dissipation by Hb and HbO_2 electronic excitation energy. The heat generated in this process is transferred to the blood capillaries, which has the characteristic time of thermal relaxation ~ 0.05-1.2 msec.

The mechanism of the laser-tissue interaction is very much dependent on the output laser energy. The effect of high-energy lasers is quite clear and based on photothermal processes such as selective photothermolysis.

This mechanism is used in clinical practice, for example, in laser surgery, cosmetology, laser correction of vision etc. It is clear that the effect of heating due to absorption of low energy laser radiation in a tissue is negligible. Estimate shows that in typical case the local increase of temperature only by 0.1 - 0.5 ^{0}C may be expected. Such a small raise of a local temperature may promote only some improvement in capillary microcirculation of blood and hardly could stimulate the metabolism of cells.

We suppose that in a case of low energy lasers the most important process is the photodissociation of HbO_2, whose main biological function is the transport of molecular oxygen. The quantum efficiency of the photodissosiation [11] of oxyhemoglobin is amazingly high and reaches 10 % in a wide visible spectral range. The molecular oxygen is generated due to laser-induced photodissosiation of HbO_2 in blood vessels allows control the local increase of oxygen concentration at irradiating region (fig. 1).

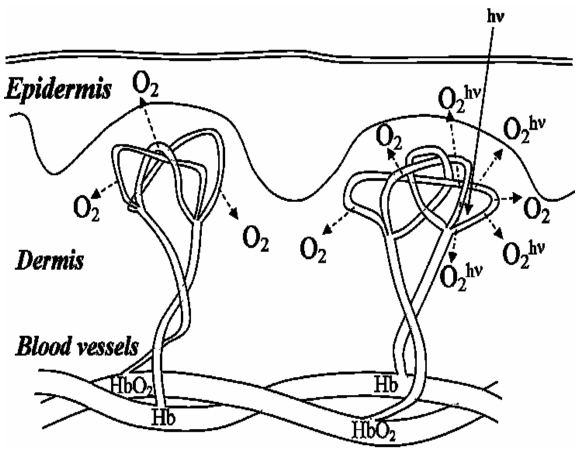


Fig. 1 - Illustration of laser-induced tissue oxygenation caused by photodissociation of arterial blood HbO_2

The possibility of additional oxygen supply allows develop a new method of tissue hypoxia elimination that restores normal cell metabolism. Investigation of photodissociation of hemoglobin complexes in vivo could be carried out using arterial blood saturation parameter.

In case of HbO_2 the value of saturation SaO_2 in arterial blood vessels is defined by the concentration of HbO_2 taking into account contribution of Hb, methemoglobin (MetHb) and carboxyhemoglobin (HbCO).

$$SaO_2 = \{[HbO_2] / ([HbO_2]+[Hb]+[MetHb]+[HbCO])\}100$$

At normal conditions of gas exchange the concentrations of MetHb and HbCO are extremely low (0.2 - 0.6 % and 0.8 % correspondingly) so the contribution of these components can be neglected. Thus in practice the value of SaO_2 could be determine as

$$SaO_2 = \{[HbO_2] /([HbO_2]+[Hb])\}100$$

Photodissociation of HbO_2 induced by laser radiation releases free molecular oxygen. Meanwhile, proportion between $[HbO_2]$ and [Hb] concentrations is changed that decrease the value of SaO_2.

$$\Delta SaO_2 = SaO_2 - SaO_2^{h\nu}$$

Were SaO_2 is saturation without and $SaO_2^{h\nu}$ with laser irradiation.

Amount of oxygen available for cell metabolism delivered by microcirculation is the function of:

$$\sum O_2\,(TcPO_2) = f(F(HbO_2)*[O_2])$$

Were HbO_2 is the value of oxyhemoglobin arterial blood and $[O_2]$ - is the concentration of oxygen released into plasma.

In the case of deterioration of the blood microcirculation extra oxygen supply is critical to provide the demands of cell for normal metabolism. This could be reached by in vivo laser-induced photodissociation of HbO_2 directly at the zone were necessary to increase the local concentration of free molecular oxygen.

As a result we obtain average concentration of oxygen that releasing in conventional way and due to

$$\Sigma[O_2] = [O_2] + [O_2^{h\nu}]$$

Thus phenomena of laser-induced in vivo photodissociation of oxyhemoglobin in cutaneous blood vessels and capillaries gives unique possibility of optically increase the local tissue oxygen concentration.

III. REGISTRATION OF BLOOD OXYHEMOGLOBIN PHOTODISSOSIATION IN VIVO

Experimental study the change of arterial blood saturation due to laser-induced photodissociation of oxyhemoglobin is based on registration the variations of its value on the background natural oscillations of saturation. Specialized pulse oxymeter spectrophotometer for recording photoplethysmogram with high accuracy and detailed numerical signal processing has been applied. Despite of traditional pulse oxymeter instead of two channels for signal registration in red and infrared spectral ranges fore channel that supplied parallel 8 independent signal processing it has been used [12,13]. As a result the registration of small changes of arterial blood saturation for one heart pulse is reached with accuracy less than 0.5 %.

The measurements of the value SaO_2 was carried out with the high sensitive pulse oxymeter sensors in transmitting light with accuracy better than 0.5 %. The sensor was placed on the first of the two phalanxes of the finger and measuring elements were in the region of the first phalanx (fig. 2).

The effect of laser radiation on arterial blood oxygen saturation has been observed using He-Ne laser with wavelength $\lambda = 632$ nm, which is mostly applied in medical practice. Lasers spot on a skin was about 7-8 mm with power density of 20 mW/cm^2. The laser radiation was guided to an interior of the third phalanx of a finger.

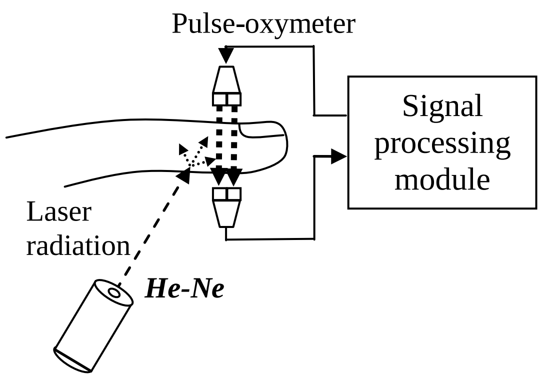


Fig. 2 - Experimental setup for investigation of the effect of laser radiation on the value of arterial blood saturation

The concept of laser-induced tissue oxygenation allows understand the mechanism of biological response and therapeutic effect of laser radiation. Its also gives a unique method of selective local tissue oxygenation, that could be used in wide range of biomedical applications.

Using another direct method of oximetry (Fig. 3) based on principle of measuring the oxygen tension PO_2 in arterial blood is direct method of registration of gas that dissolved in blood plasma.

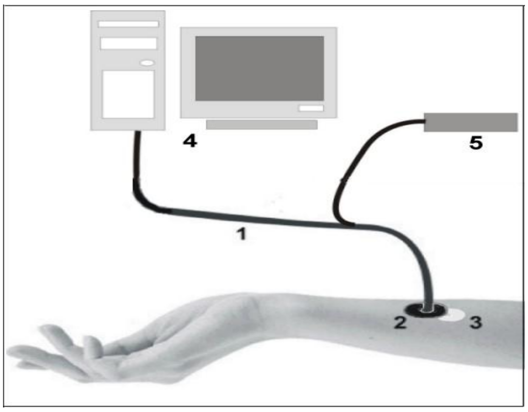


Fig.3 - Measurement of tissue oxygen tensions due to photodissociation of blood HbO_2: 1 - Clark sensor, 2 - electrolytic cell, 3 - irradiating zone, 4 - monitor TCM - 4, 5 - He- Ne laser.

For this usually are used Clark-type polarographic sensor ("$TcPO_2$ electrode", see fig. 3) that consist of silver anode, electrolyte, and an oxygen permeable membrane; heating section and electronic system for measuring and controlling the sensor temperature.

The initial oxygen tension in tissue was measured by placing $TcPO_2$ electrode on human skin in shoulder area. Then He-Ne laser radiation at the power of 1mW was applied. Kinetics of tissue oxygen tension was experimentally investigated [14]. Obtained results were normalized to initial oxygen tension value.

These two above mentioned methods allows to measure and control the process of releasing extra oxygen from HbO_2 under laser irradiation directly to arterial blood plasma and future it diffusion into tissue.

IV. NEW METHOD OF OPTICAL "DOSIMETRY" BASED ON CONTROLLING LOCAL TISSUE OXYGANATION

Laser-induce photodissociation of HbO_2 gives a novel and unique method for optically increasing the local concentration of free molecular oxygen in tissue that is significantly enhances cell metabolism. Taking into account that blood deliver O_2 to any cell tissue and metabolism of cells required consumption of oxygen we suggest to base therapeutic effect of laser radiation based on controlling summary tissue oxygen concentration.

- Proposed method for optimization therapeutic efficiency the effect of laser radiation is based on using the change in oxygen concentration as feedback signal.
- Oxygen released into tissue is proportional to the energy of aerobic cell metabolism.
- Photodissociation of HbO_2 increases the level of tissue oxygenation.
- Oxygen release rate could be directly measured in vivo through the value of saturation - SaO_2
- Capacity of circulatory system to carry oxygen defined by hemoglobin concentration [Hb] and also is a function of how much blood per minute is pumped from the heart.

Controlling parameters are:

- Aerobic metabolism - (energy production);
- Extra oxygen release into tissue due to photodissociation of HbO_2;
- Ability of blood circulation system to transport oxygen.

Measuring parameters:

- Amount of oxygen released into tissue;
- Changes in arterial blood saturation $DSaO_2$;
- Hemoglobin concentration and heart pulse rate.

In this case we can refer to the pulse volume of heart V_H which is equal to blood volume in liters that pump the heart at one bit. Then oxygen flux $F(O_2)$ through the irradiating zone of tissue we can describe as

$$F(O_2) = 4[O_2]/ ([Hb] + 4[O_2]) * C * V_H * [Hb]* (SaO_2 / 100),$$

Where C – is a coefficient of blood delivery to tissue indicating tension in capillary blood vessels and [Hb] – is the concentration of hemoglobin in gm/l. SaO_2 – is the degree of hemoglobin oxygen saturation in percents, and $[O_2]$ – molar concentration of oxygen.

Than we introduce the notion of "standard flux of oxygen through the tissue"

$$S(F.O_2) = 4 [O_2] / ([Hb] + 4 [O_2]) * [Hb]_n* C * V_H$$

"Standard flux of oxygen through the tissue" indicates a flux of oxygen that is necessary for supplying tissue at normal conditions.

Normal conditions are related to the concentration of hemoglobin in blood that corresponds for the given age and complete saturation with oxygen.

For the estimation of current oxygen delivery through the tissue we normalize the local flux to the standard one

$$F(O_2) = F(O_2) / SF(O_2)$$

Than we obtain

$$F(O_{2)} = [Hb]/[Hb]_n* (SaO_2 / 100)$$

This parameter allows us estimate current efficiency of oxygen delivery in dependence of the concentration of hemoglobin and degree of its saturation with oxygen. Now we can determine the quantity of oxygen that releases into tissue during elimination with low intensity laser radiation.

$$\Delta FO_2 = F(O_2) - F(O_2)^{h\nu}$$

Were FO_2 - is normalized flax of oxygen without laser irradiation and $FO_2^{h\nu}$ - is normalized flax of oxygen during laser irradiation.

The dose of oxygen that that releases into tissue during elimination with low energy laser radiation can be determined from following expression:

$$[O_2] = \{F(O_2) - F(O_2)^{h\nu}\} * T * Pr$$

Were T – is the time of irradiation and Pr - is pulse rate.

Substituting expressions for FO_2 and $FO_2^{h\nu}$ we obtain:

$$[O_2] = ([Hb]/[Hb]_n * (SaO_2 / 100) - [Hb]/[Hb]_n * (SaO_2^{h\nu} / 100)) * T * Pr =$$

$$= T * Pr * [Hb]/[Hb]_n * (SaO_2 - SaO_2^{h\nu}) / 100 = T * Pr * [Hb]/[Hb]_n * (\Delta SaO_2 / 100)$$

Thus the "dose" of oxygen that that releases into tissue during irradiation with low intensity laser radiation can be determined from following expression:

$$\Sigma[O_2] = T * P_r * [Hb]/[Hb]_n * (\Delta SaO_2 / 100),$$

Suggested method of determination of therapeutic dose of laser radiation correlated with tissue local oxygenation could be applied in clinical practice. Developed high sensitive pulse oxymeter completely provides determination of all parameters for establishing a therapeutic dose for healing a huge variety of diseases by laser phototherapy.

Laser induced photodissociation of HbO_2 allows extract additional amount of oxygen locally at irradiating zone. This phenomenon provides unique possibility using optical methods for regulation of local tissue O_2 concentration. Additional oxygen release rate is directly measured through the value of oxyhemoglobin arterial blood saturation (ΔSaO_2). The amount of oxygen released into tissue depends also from capacity of circulatory system to carry oxygen.

This capacity mainly defined by contribution of two following parameters: hemoglobin concentration in blood [Hb] and its circulation speed. The impact of actual hemoglobin concentration is described by the ratio of $[Hb]/[Hb]_n$. Were $[Hb]_n$ is standard concentration that is normal for particular sex and age. The impact of blood circulation speed is taken into account through the heart pulse rate P_r.

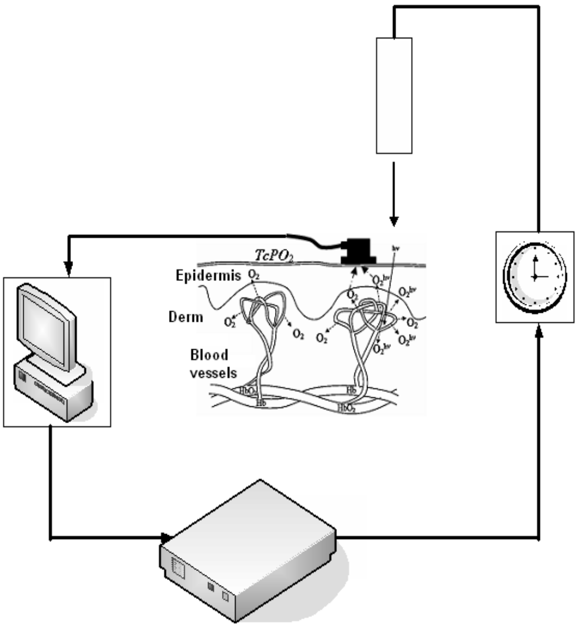
Finally, therapeutic "dose" can be determine by the value of $\Delta SaO_2 = SaO_2 - SaO_2^{h\nu}$, were SaO_2 is saturation without and $SaO_2^{h\nu}$ with laser irradiation, heart pulse rate P_r, time of exposure T, ratio of actual and standard hemoglobin concentrations $[Hb]/[Hb]_n$.

$$D(O_2) = \frac{[Hb]}{[Hb]_n} \cdot \left(\frac{\Delta SaO_2}{100} \right) \cdot P_r \cdot T$$

It should be noted that involved parameters are objective and could be measured by well-known clinical routine. Suggested new method of optical "dosimetry" based on key biological parameters and connected with aerobic cell metabolism provides possibility of precise determination of therapeutic effect of laser radiation.

V. EXPERIMENTAL

Experimental investigation the phenomenon of laser-induced tissue oxygenation has been carried out using transcutaneous oxygen monitor (TCOM) - "Radiometer" TCM-4 (Fig.4).



concentration directly at the zone of laser irradiation

Direct in vivo measurements of tissue oxygen tension $TcPO_2$ under irradiation by He-Ne laser at the power of 1mW has been carried out [14].

Using the simple diffusion model we calculated what amount of oxygen should be released into blood plasma in order to reach experimentally observed increasing tissue O_2 concentation (fig. 5). The target criteria were kinetic of tissue oxygenation in response to laser irradiation.

The variable parameters were diffusion coefficient of oxygen in tissue and oxygen release rate.

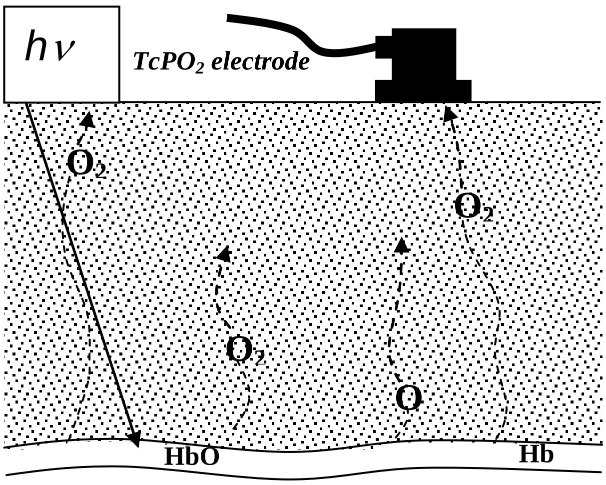


Fig. 5- Simple model of oxygen diffusion in tissue

As it was shown experimentally [10] the response of oxygen release on laser irradiation is relatively fast and remains constant during the irradiation. To simulate this effect in the model, the oxygen release rate was increased instantly and remains constant during the time of irradiation.

The main aim of the calculation was to reach best fit of the data produced by the model to the experimentally measured one. The target criteria were kinetic of tissue oxygenation in response to laser irradiation. The variable parameters were diffusion coefficient of oxygen in tissue and oxygen release rate.

VI. RESULTS AND DISCUSSION

The kinetic of oxygen tension in tissue in two cases the normal blood circulation and artificially induced ischemia was investigated. Obtained results were normalized to initial oxygen tension value.

In fig. 6 the results of cold laser induced tissue oxygenation in the case of artificially induced ischemia are presented. As it seen we still can extract extra oxygen from arterial blood and optically supply the demand of cell metabolism as long as needed.

As it seen from fig.6 during laser irradiation the value of tissue oxygenation is increases exceeding its initial level about 1.6 times (curve 1) after ten minutes of illumination. In the case of induced ischemia additional extraction of oxygen also is observed. This result clearly demonstrates that laser-induced tissue oxygenation could be applied in clinical practice for restoration of normal cell metabolism in tissue with damaged microcirculation.

The results of calculations demonstrate that in order to reach experimentally observed the rise of $TcPO_2$ by 1, 6 times at the surface of tissue, the calculation indicates the increase of oxygen release rate from arterial HbO_2 into blood plasma should increase about 4,3 times.

Photodissociation of HbO_2 induced by laser radiation and release rate of free molecular oxygen into blood plasma has been measured experimentally in vivo using high sensitive pulse oxymeter. The oxygen released from HbO_2 primarily increases the PO_2 of blood plasma and then O_2 diffuses into a tissue.

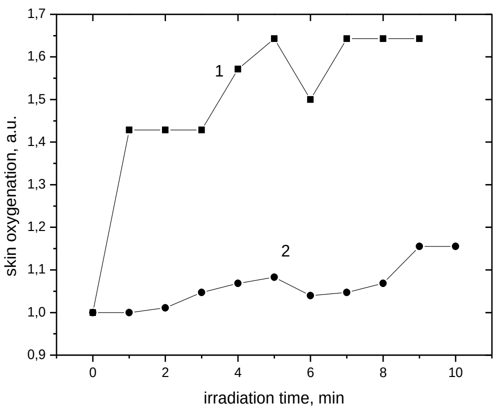


Fig. 6 - The kinetics of laser-induced tissue oxygenation during laser irradiation in norm blood microcirculation -1, and in artificially induced ischemia - 2

It is exiting that the value of PO_2 in blood plasma reached by laser-induced photodissociation of HbO_2 is comparable to that one typically reaches by the method of HBO. The distribution of $TcPO_2$ in the volume at the irradiation zone is depended on the time of exposure and the properties of tissue.

The comparison of calculated results with experimental data demonstrates that kinetic of $TcPO_2$ in dependence of time of elimination by laser radiation gives possibility to determine O_2 diffusion coefficient into tissue. This means that one could calculate and determine how to reach desirable level of $TcPO_2$ in zones with the disturbed blood microcirculation such as solid tumor, burn or wounds. So it's possible to determine optimal parameters of irradiation taking into account the volume that has to be oxygenated and the time of elimination.

Thus our suggested novel method can eliminate the deficit of oxygen until the restoring new vascular net in tissue. This result could be applied in the case of those pathologies where elimination of tissue hypoxia is critical.

Supplemental oxygen can lead to increased rate of collagen deposition, epithelialization and improved healing of split thickness grafts. Increased subcutaneous TcPO2 has also been shown to improve bacterial defenses. Thus unique possibility in selective and local increase of the concentration of free molecular oxygen into tissue that enhances metabolism of cells is developed. Laser-induced enrichment of tissue oxygenation stimulates cell metabolism and allows develop new effective methods for laser therapy as well as phototherapy of pathologies where elimination of local tissue hypoxia is critical.

Laser-induce photodissociation of HbO_2 may serve as a unique method in laser therapy for optically increasing the local concentration of free molecular oxygen in tissue that is significantly enhances cell metabolism.

It is valuable that even at the case of ischemia we still can extract extra oxygen from arterial blood and optically supply the demand of cell metabolism as long as needed. Thus laser-induced tissue oxygenation allows optically eliminate the deficit of oxygen until the restoring new vascular net in tissue.

Obtained results gives an experimental argumentation to consideration of primary mechanism of biostimulation and therapeutic effect of low energy laser radiation that could be based on increasing tissue local oxygen concentration directly wt the zone of irradiation.

This phenomenon allows to develop an objective method of control the efficiency of treatment by laser phototherapy. Now in clinical application the parameters of laser radiation can be tuned to optimal wavelength, power and exposition time in depends of optical characteristics of the patient skin tissue.

The obtained results also shows the way of increasing the efficiency of biostimulation and therapeutic effect of low energy laser radiation based on combination it with method of oxygen hyperventilation therapy.

An important conclusion can also be drawn from the obtained results. In interpretation of the biostimulating and heeling effect of laser radiation the phenomenon of induced photodissociation of blood oxyhemoglobin should be taken into account.

VII. CONCLUSION

New optical method of elimination the local tissue hypoxia is developed. The value of tissue oxygen concentration increases significantly during the laser irradiation.

It is shown that therapeutic dose of laser radiation could be based on adjusting the local concentration of free oxygen in tissue by laser-induced photodissociation of blood oxyhemoglobin.

To make the phototherapy as well as laser therapy methods really efficient one has to control the oxygen concentration in tissue keeping it at the necessary level. This goal could be reached by the use of laser-induced photodissociation of oxyhemoglobin in tissue blood vessels.

Method of determination of oxygen diffusion coefficient into tissue based on kinetics of tissue oxygenation under the laser irradiation is developed.

It is shown that the efficiency of laser-induced oxygenation is comparable with the method of hyperbaric oxygenation (HBO) at the same time gaining advantages in local action.

Novel method of optical “dosimetry” based on using the changes in tissue oxygen concentration as feedback signal for optimization therapeutic effect of low intensity laser radiation is developed.

Photodissociation of oxyhemoglobin, whose main biological function is the transport of molecular oxygen gives unique possibility of additional oxygen supply and allows develop laser-optical method of tissue hypoxia elimination that restores normal cell metabolism.

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