

## BIOMEDICAL APPLICATION OF LASER-INDUCED TISSUE OXYGENATION

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### Abstract

*The results of investigation of laser-induced photodissociation of oxyhemoglobin in cutaneous blood vessels and its role in biomedical processes are presented. We propose a new method for determination an individual response to the effect of laser radiation. It is shown that in order to make the phototherapy 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.*

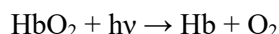
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### 1. INTRODUCTION

Biophotonics of "laser-tissue" interaction are considered as one of the interesting aspects of modern photomedicine and photobiology. 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 make difficult to develop correct method for controlling the efficiency of laser therapy - correct "dose" of delivered average energy of laser radiation [1,2].

Tissue oxygenation plays a key role in metabolism, energy supply, and life activity of an organism. Controlling this mechanism gives the possibility of biological stimulation to achieve therapeutic effect. Factors that can affect the delivery of oxygen in tissue are of considerable interest, and one of them is the light radiation in the optical spectral range.

Laser-tissue interaction and especially the light's impact on the hemoglobin complexes in the cutaneous blood vessels can be considered as one of the interesting aspects of modern photomedicine. A new approach for optically induced increase in the local oxygen concentration due to photodissociation of oxyhemoglobin in cutaneous blood vessels has been suggested in [3]. The absorption of light by blood HbO<sub>2</sub> is connected with the photochemical and photophysical processes. It is known, that the absorption of a photon with the activation energy leads to dissociation of a part of the molecules resulting in release of molecular oxygen and formation of deoxyhemoglobin. Photodissociation is one of the simplest chemical reactions when a compound dissociates under the action of radiation.



Due to phenomenon of photodissociation additional amount of molecular oxygen could be extracted directly in the zone of laser irradiation. Actually, the quantum efficiency of the photodissociation of HbO<sub>2</sub> is high and reaches 10 % in a wide spectral range. Molecular oxygen extracted due to laser-induced photodissociation of HbO<sub>2</sub> in cutaneous blood vessels allows control of the local tissue oxygen concentration. The efficiency of this process depends on the wavelength and the output power of the applied laser radiation, the optical properties of the skin and the depth of the blood vessels in the tissue.

It is reasonable to expect that the in vivo photolytic effect will be different from that observed in buffer solutions, considering the different biological environment and specific features of light propagation in biological tissue.

In this paper 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.

## 2. MATERIALS AND METHODS

Investigation of photodissociation of hemoglobin complexes in vivo could be carried out using arterial blood saturation parameter arterial oxygen saturation ( $SpO_2$ ). By definition,  $SpO_2$  is determined as the ratio of oxygensaturated hemoglobin ( $HbO_2$ ) to the total concentration of hemoglobin (unsaturated  $Hb$ +saturated  $HbO_2$ ) in the blood, i.e., the  $SpO_2$  determines the relative  $HbO_2$  concentration in blood.

Determination of  $SpO_2$  concentration in the blood is performed by a method similar to the method of pulse oximetry, which is based on the measurement of light-modulated pulse wave of blood. The values are measured using a pulse 3-wavelengths sensor operating in backscattered light, based on a standard pulse oximetry pair of V97B light emitting diodes ( $\lambda = 660$  nm and 935 nm) and L-53MGC light emitting diode ( $\lambda = 568$  nm) and a BPW34 silicon photodiode. Due to our original method of data processing [4] the designed 3-wavelengths measurement system provides a maximum difference in the absorption coefficients of different derivatives of hemoglobin and makes it possible to obtain a reliable measuring of the  $HbO_2$  level with high accuracy ( $< 0,2\%$ ). The system allows continuous photoplethysmographic monitoring, recording and data storage.

The pulse oximetry sensor is placed on the first phalanx of the finger (see Fig.1). The light was delivered to the finger from LEDs (or laser diodes) at fifteen wavelengths in the 400–940 nm spectral range. The optical power of every light source is selected to provide an approximately equal number of photons of different wavelengths incident on the irradiated skin area. The corresponding power density of irradiation taking into account the output aperture varied from 50 mW/cm<sup>2</sup> for  $\lambda = 405$  nm to 125 mW/cm<sup>2</sup> for  $\lambda = 940$  nm.

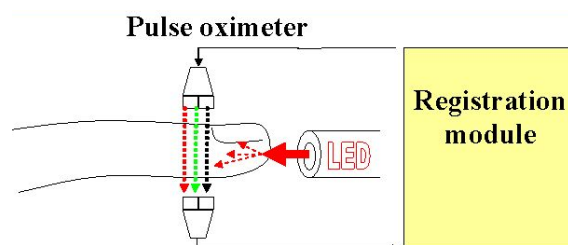


**Fig. 1.** Experimental set up. Measuring of  $HbO_2$  in vivo by pulsoximetric system

The irradiation beam with diameter 4 mm is directed at the lower front of the phalanx, at about 5 mm distance from the measuring sensor, so that direct light does not reach the surface of the photodetector. In order to eliminate rogue results, only measurements which were within the range between  $\pm 20\%$  standard deviations from the mean were included in the analysis. All results are given as mean  $\pm$  (SD). The Student's test for connected sampling was used for the statistical calculations. The evaluation was carried out using the program "Origin 7.5".

## 3. RESULTS

In vivo investigation of the effect of different laser wavelengths on the efficiency of  $HbO_2$  blood photodissociation was carried out experimentally. Each light source was recorded from 10 to 25 sets of three-wave photoplethysmogram as follows: 30-40 s with no radiation, 30-40 s with radiation and 30-40 s without radiation. Photoplethysmograms are recorded for all wavelengths and results of the

experiment are averaged. Fig. 2 shows a typical example of changing the rate of the SpO<sub>2</sub> level in the blood measured with and without LEDs exposure on the first finger phalanx (in this case 635 nm).

As can be seen, the changes of saturation take place immediately when illumination is started at 40 s and the level of saturation does not depend on the irradiation time. The effect disappears with stopping the illumination and saturation restores up to its initial value. These results clearly demonstrate that the observed effect is a local and prove the effect of laser induced photodissociation of oxyhemoglobin in blood capillary vessels. During irradiation the SpO<sub>2</sub> level remains constant and returns to initial value practically immediately after the irradiation shutdown. It is evident that the observed local drop of arterial oxygen saturation during irradiation is caused by HbO<sub>2</sub> photodissociation in capillary blood vessels.

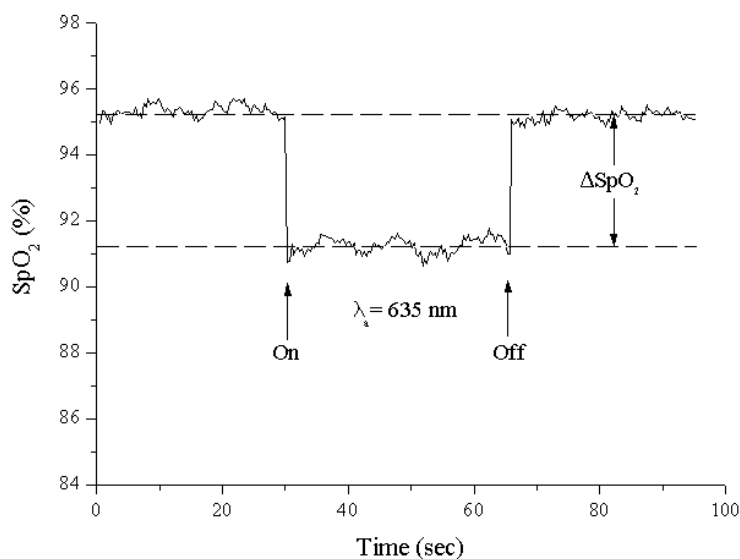


**Fig. 2.** Changes in level of SpO<sub>2</sub> caused by irradiation of the first finger phalanx at 635 nm

Using described above technique and method we experimentally measured the laser-induced photodissociation of HbO<sub>2</sub> in cutaneous blood vessels in the visible and near IR spectral range. We determine the parameter  $\Delta\text{SaO}_2$  as the difference between the initial values of SaO<sub>2</sub> before irradiation and the measurable values in the experiment. The initial values of relative SaO<sub>2</sub> varied from 95 % up to 97 %. Using statistical analysis of measurement data obtained for all sources of radiation the parameter  $\Delta\text{SaO}_2$  was calculated.

The results demonstrate that the rate of the SaO<sub>2</sub> in blood varies under the influence of local transcutaneous light irradiation and significantly depends on the radiation wavelength. Three absorption maxima in the spectral range (near 530, 610 and 850 nm) are revealed (see Fig. 3).

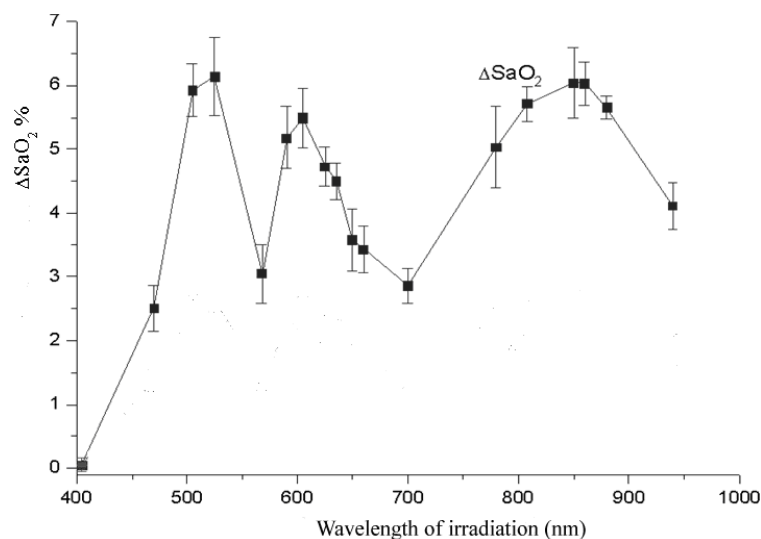


**Fig. 3.** Change in blood saturation (%) depending on the irradiation wavelength.

The results obtained correlate with the absorption spectrum of HbO<sub>2</sub> and the transmission spectrum of the skin. In reviewing the obtained spectrum, features of the propagation of light deep into the tissue should be considered. The visible light is more absorbed in the superficial layers of the skin, so the efficiency of absorption at  $\lambda = 530$  nm and  $\lambda = 600$  nm remains equal with the efficiency at  $\lambda = 860$  nm only up to 2-3 mm of the tissue depth. In the deeper layers (more than 5 mm) of tissue the IR band of the HbO<sub>2</sub> plays a dominant role in the absorption of laser radiation. Furthermore, the skin is more transparent for the IR range. In medical practice different semiconductor lasers (820 nm, 870 nm, 880 nm and 904 nm) are widely used for wound healing and treatment of various diseases.

#### 4. DISCUSSION

In contrast to other in vitro studies, this study was carried out under in vivo conditions, so it is possible to make statements about the practicability of the measurement effect.

Thus, we demonstrate that the rate of the SpO<sub>2</sub> in blood varies under the influence of local transcutaneous LED irradiation and significantly depends on the radiation wavelength. The results obtained correlate well with the calculated absorption spectra. It is evident that the observed local decrease of arterial SpO<sub>2</sub> during irradiation is caused by HbO<sub>2</sub> photodissociation in blood capillary vessels. The fact that the saturation returns to initial value practically immediately when the illumination is switched off can be explained by the fast geminate recombination and oxygen rebinding as well as by blood evacuation from the site of measurement due to blood flow.

The results obtained correlate with the characteristics of light propagation in blood filled tissue. The visible light at 450 nm and 580 nm has a small penetration depth into skin tissue, because of their proximity to the absorption bands of some basic skin chromophores such as oxyhemoglobin and melanin. Red and especially near IR radiation penetrate much deeper into soft tissues. Therefore irradiation in the region of the  $\alpha$ - and  $\beta$ -bands, not to mention Soret band, which can be effective for very thin blood layers, in real tissue can cause HbO<sub>2</sub> photodissociation only in shallow superficial skin layers.

Therefore, it is clear that under light irradiation of more or less considerable tissue volume ( $\geq 1$  cm<sup>3</sup>), the primary role in HbO<sub>2</sub> photodissociation is due to red and especially near IR radiation.

Thus, we can expect that after prolonged irradiation (several minutes), a certain fraction of oxygen molecules released due to photodissociation of HbO<sub>2</sub> will diffuse in the surrounding tissue and increase the oxygen partial pressure.

Such possibility to increase the free oxygen content in tissues can be applied in clinical practice for treatment of a number of diseases related to problems with the microcirculation and oxygen supply.

## 5. BIOMEDICAL APPLICATIONS OF LASER-INDUCED TISSUE OXYGENATION

The concept of laser-induced tissue oxygenation allows understand the mechanism of biological response and therapeutic effect of laser radiation. It also gives a unique method of selective local tissue oxygenation, that could be used in wide range of biomedical applications. Short overview of some promising areas is presented below.

### 5.1. Phototherapy of wounds and burns

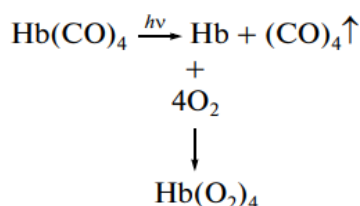
Deficit of oxygen makes it difficult to treat wounds and burns, bedsores [5]. It has been proven that the effectiveness of the therapy of the listed pathologies strongly depends on the oxygen pressure  $PO_2$  in the tissues. The proposed method of laser-induced tissue oxygenation can be successfully applied as an adjunct in the combined treatment of similar pathologies, supplying additional amounts of oxygen. For example, adequate supply of tissues with oxygen and maintenance of  $PO_2$  at the level of 40 mm Hg and higher accelerates the synthesis of collagen and elastin, which plays a key role in wound healing. It is interesting to note that photodissociation of oxyhemoglobin and additional release of molecular oxygen can also be achieved by laser-induced photodissociation of  $HbO_2$  under intravenous blood irradiation.

### 5.2. Phototherapy of anaerobic infections

Oxygen is an important component in fighting the infections that one is afflicted with. It is obvious that a molecule of oxygen released from hemoglobin in the presence of bacteria 'burns' up the bacteria. The white blood cells in the human body have a similar action. They flow through the circulation system and encounter bacteria or other foreign matter. Once an object is encapsulated within the white blood cell, it is bathed in superoxidase compounds and is destroyed or 'burned' up. Without this, the white blood cells, though effectively attacking the bacterial agents, are not able to break down and destroy the bacteria. The red blood cells, under normal circumstances, can carry more than enough oxygen to fuel the body cells. If a passing white blood cell needs oxygen, the major component is a superoxidase compound that is able to pull it from a passing red blood cell. This method is very efficient, however, in the case of extreme anemia the quantity of the red blood cells may not be enough for extracting the oxygen to fight infection. This makes the monitoring of the oxygen concentration very important in therapy. Thus, suggested method of laser-induced tissue oxygenation and its dosimetry could be successfully applied in treatment the toxicity of pathogenic different anaerobic bacteria.

### 5.3. Phototherapy of carbon monoxide poisoning

Carbon monoxide (CO) is high toxic odorless and colorless gas. The toxic effect of CO exerts through tissue hypoxia [6]. The hemoglobin (Hb) molecule has an affinity for CO that is 240 times greater than that for oxygen ( $O_2$ ). The formation of carboxyhemoglobin ( $HbCO$ ) leads to a reduction the concentration of oxygen that is delivered by oxyhemoglobin ( $HbO_2$ ) to the vital organs and tissues. The results obtained allow us to propose an approach for increasing the effectiveness of carbon monoxide decomposition. It is shown that the significant difference in the quantum yields of photodissociation of hemoglobin complexes makes possible a selective decomposition of  $HbCO$  in the blood stream with minimum effect on the  $HbO_2$  component.



The rate of CO photodissociation from the HbCO molecule is extremely high and its quantum yield, which is almost 80%, allows us to expect high efficiency of the proposed approach in clinical applications. Maximum photodissociation of HbCO can be reached in the lungs alveolus or in cutaneous blood vessels by irradiating at wavelengths near 530, 600 and 850 nm, which are in the region of the maximum effect in the visible and IR region of the spectrum. The spectral effectiveness of the photodissociation approximately correlates with the absorption spectrum of HbCO and the transmission spectrum of skin tissue. The required laser power and the treatment time in clinical practice should be determined according to the optical properties of the skin, the depth of penetration and the volume of blood, illuminated by laser radiation.

Our approach, based on photodissociation of the blood HbCO, in combination with other existent methods (oxygen hyperventilation, as well as hyperbaric oxygen therapy) significantly increases the efficiency of CO poisoning treatment. An important conclusion is that there is a possibility of laser-induced reduction of HbCO in the blood. The distinctive feature of this technology is its selectivity and local action.

#### 5.4. Photodynamic therapy

It is well known that deficit of oxygen in cancer tissue is the major factor that limits the efficiency of the method of photodynamic therapy (PDT) [7]. There are two factors determining hypoxia in cancer tissues. First is the fast growth of cancer cells which leads to disordering angiogenesis. Second factor is connected with the rapid decrease of the free oxygen concentration in cancer tissue due to the generation of singlet oxygen in PDT. Laser-induced photodissociation of HbO<sub>2</sub> can be used for increasing the efficiency of the method of PDT. As soon as molecular oxygen is a major component of photodynamic reaction the efficiency of PDT could be significantly improved by applying laser-induced photodissociation of HbO<sub>2</sub> with increasing the local temperature of tissue.

## 6. CONCLUSIONS

Obtained results gives an experimental argumentation to our consideration that therapeutic effect of low energy laser radiation is based on local increase of the concentration of oxygen due to induced photodissociation of oxyhemoglobin in cutaneous blood vessels. A possibility in selective and local increase of the concentration of free molecular oxygen in tissue that enhances metabolism of cells is demonstrated. The rates of SaO<sub>2</sub> in blood in vivo and their dependence on the wavelength of the transcutaneous laser irradiation as well as from the irradiation power and the tissue temperature have been experimentally measured. The observed reduction in SaO<sub>2</sub> up to 10% indicates the process of photodissociation of HbO<sub>2</sub> in vivo and may result in the local O<sub>2</sub> increase in the tissue. This approach gives the possibility for additional oxygen supply and allows development of optical methods for tissue hypoxia elimination. In interpretation of the biostimulating and healing effect of laser radiation the phenomenon of induced photodissociation of blood oxyhemoglobin should be taken into account.

## ACKNOWLEDGMENTS

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