Direct laser-induced singlet oxygen in biological systems: application from *in vitro* to *in vivo*

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***Abstract*—In recent years, there has been increasing interest in the singlet form of oxygen as a regulator of the physiological functions of cells. The use of photosensitisers is a classical mechanism for the excitation of the main triplet form of oxygen and the generation of its singlet form. At the same time, it has been shown that there is a possibility of direct optical excitation of the main oxygen form into the singlet state by light at certain wavelengths. This review article aims to combine recent accumulated experience in the field of direct optical generation of singlet oxygen. We focus on works on the application of a 1267 nm wavelength, which is the most frequently used and well-studied in this area. In this review, we consider the use of laser-induced singlet oxygen in various biomedical applications both at the cellular level and at the level of whole organisms. This review presents the latest results on the use of singlet oxygen for therapeutic effects on cancer cells, as well as for photostimulation of neurons and the vascular and lymphatic systems.**

***Index Terms*—singlet oxygen, direct optical generation, cancer, cardiovascular system, ATP production, photostimulation**

I. Introduction

OXYGEN and reactive oxygen species (ROS) play a significant role in the regulation of basic functions of the cell, both under normal conditions and under the influence of various pathogenic factors. Oxygen is a strong oxidiser, which makes it an excellent electron acceptor in the mitochondrial respiratory chain. At the same time, ROS are formed as necessary intermediates in oxidation reactions [1]. ROS are oxygen ions, free radicals and peroxides of both inorganic and organic origin. These are usually small molecules with exceptional reactivity due to the presence of an unpaired electron at the external level. They are constantly formed in a living cell, being products of physiological oxygen metabolism or the result of external influence. In recent years, the active study of the physiological role of ROS has gathered special interest in the singlet form of oxygen. Singlet oxygen ($^1O_2$) is a less stable electronically excited state of triplet oxygen ($^3O_2$) and is produced in various ways, including thermal, enzymatic or photochemical activation of O_2.

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The classical mechanism for the excitation of the main triplet oxygen form and the generation of its singlet form is the use of photosensitisers (PS), which, under the influence of light, transfer the electronic excitation energy to triplet oxygen [2]–[4]. These compounds have a relatively long excited-state lifetime, which allows them to interact effectively with triplet oxygen and transfer their energy to it. However, many PSs have drawbacks regarding their incorporation or interaction with living systems (poor absorption, uncontrolled localisation in cells, induction of cellular stress, direct cytotoxicity, etc.).

At the same time, it has been shown that there is a possibility of direct optical excitation of the main oxygen form into the singlet state by light at certain wavelengths [5]. The first works on the biological and clinical application of direct optical generation of $^1O_2$ date back to the 1990s. For example, S.D. Zakharov and A.V. Ivanov investigated biological effects when exposed to a 1270 nm laser [6]. They evaluated the effect on the refractive index and the change in the cell membrane of the erythrocyte suspension and demonstrated the possibility of using this wavelength for the treatment of tumours in mice. Further, in a series of publications, A.A. Krasnovsky et al. additionally substantiated the real possibility of achieving $^1O_2$ generation by direct optical illumination [7]–[9]. Since 2010, extensive studies have been carried out on direct optical excitation of $^1O_2$ [5]. In particular, this was facilitated by the development of quantum-dot laser diodes that emit in the near-infrared (NIR) spectral range [10]. Their emission wavelength centred at around 1267 nm coincides well with the NIR absorption band of oxygen molecules.

Some studies in recent years show that laser-induced $^1O_2$ can lead to the formation of free radicals, mitochondrial dysfunction and cell death in cancer. In contrast, other studies demonstrate that $^1O_2$ has no toxic effect in primary astrocytes and neurons and it activates mitochondrial bioenergetics. Thus, $^1O_2$ may have a different effect on the viability of cells of different tissues. Indeed, $^1O_2$, like other ROS, is capable of having a direct destructive effect on cellular structures, as well as initiating free radical oxidation of lipids, proteins, and nucleic acids, which underlies the pathogenesis of many diseases [1]. ROS realise their physiological and pathological effects in close interaction with other regulatory factors of the cell, modulating their activity. At the same time, a lot of information has already been accumulated about the signaling role of the ROS. They can participate in the transduction of intracellular signals from various growth factors, are able to change the activity of various transcription factors [11].

Therefore, the aim of this article is to combine the ex-

perience accumulated recently in the field of direct optical generation of $^1O_2$ and to provide a review of the literature in this area. In this review, we will consider the use of laser-induced $^1O_2$ in various biomedical applications at the cellular level and at the level of whole organisms. The review will present the latest results on the use of $^1O_2$ for therapeutic effects in oncological cells and tissues, for photostimulation of neurons and the vascular and lymphatic systems. We hope that this review, based on our own experience, will be able to contribute to the development of this subject area and, in general, the development of translational research.

II. Mechanisms of Triplet-Singlet Transition

In the ground state, molecular oxygen has two unpaired electrons with parallel spins on two degenerate orbitals. This configuration gives a total spin of 3 (triplet state, $^3O_2$). When molecular oxygen acquires excess energy, it can go into a singlet state ($^1O_2$). There are two forms of $^1O_2$. The electronic configuration of these states differs only in the structure of the π-antibonding orbitals. In the ground state, there is one electron on each of the two orbitals, whose spins are parallel. In the first excited state, the electrons are paired. The configuration of the second excited state coincides with the configuration of the ground state, except that the electrons have antiparallel spins. In addition, these states differ in lifetime and, accordingly, in the effectiveness of interaction with living systems. Having paired electrons in one orbital and a vacant second orbital, $^1O_2$ has a high reactivity (several orders of magnitude greater than that of the triplet oxygen form) [12] and easily binds to electron-rich organic compounds, especially proteins, lipids, nucleic and ribonucleic acids [13]–[15]. This leads to the formation of various reactive substances, such as radicals, endoperoxides, ROS, peroxides, etc.

There is a fairly well-studied method for generating $^1O_2$ using artificial PS. This approach is currently actively used in photodynamic therapy (PDT) in the treatment of tumors [16], [17]. The most effective is considered to be the use of PDT in the initial stages of cancer. The photosensitised generation of $^1O_2$ requires only oxygen, light of a certain wavelength, and PS (see part 1 of Fig. 1) [18]. $^1O_2$ is produced as a result of energy transfer during the interaction of an excited PS with triplet oxygen. PS is transformed from the ground state (PS_0) to the excited $^1PS^*$-state after light illumination in the visible or NIR spectral range. After the intersystem crossing, the PS goes into a triplet state ($^3PS^*$) with a lifetime longer than $^1PS^*$ and can transfer energy to molecular oxygen. PS accumulates in various cell organelles (e.g., mitochondria, endoplasmic reticulum, Golgi apparatus, etc.) [19]. However, as mentioned above, this PS-based technique has a number of limitations, including uncontrolled localisation, PS toxicity, long administration time, etc.

The opportunity of direct optical excitation (PS-free excitation) of an oxygen molecule and regulation of the production of $^1O_2$ by changing the light power and exposure time is of great interest for modern redox biology and clinical medicine. The ground triplet state of oxygen has certain absorption peaks in the optical range between 390 and 1300 nm, at which $^1O_2$ can be generated (see part 2 of Fig. 1) [20]. For direct optical generation of $^1O_2$, the wavelengths of 1267, 1064 and 760 nm are most widely used [21]–[24]. Also, many studies involving the use of these wavelengths do not associate the observed effects with the generation of $^1O_2$. The latest publication [25] shows exciting results in the use of laser radiation with a wavelength of 1064 nm to modulate brain activity and improve working memory capacity. Although the authors discuss the possible increase in metabolism in brain tissues, they do not consider the underlying mechanisms. Below, in one of the

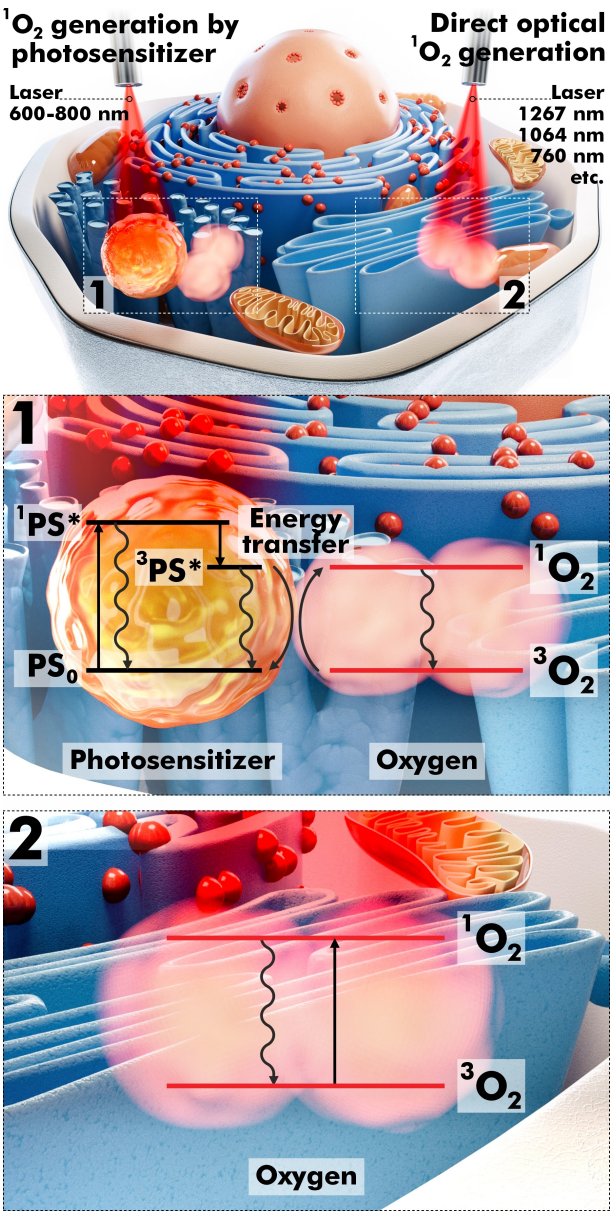

Fig. 1: Two main mechanisms of $^1O_2$ generation. (1) The photodynamic mechanism of $^1O_2$ generation by photosensitiser through energy transfer to oxygen from singlet and triplet exited states of photosensitiser ($^1PS^*$ and $^3PS^*$) and (2) the direct optical generation of $^1O_2$. 600-800 nm is the absorption range of most PS. 1267, 1064 and 760 nm are the maxima of light absorption by molecular oxygen.

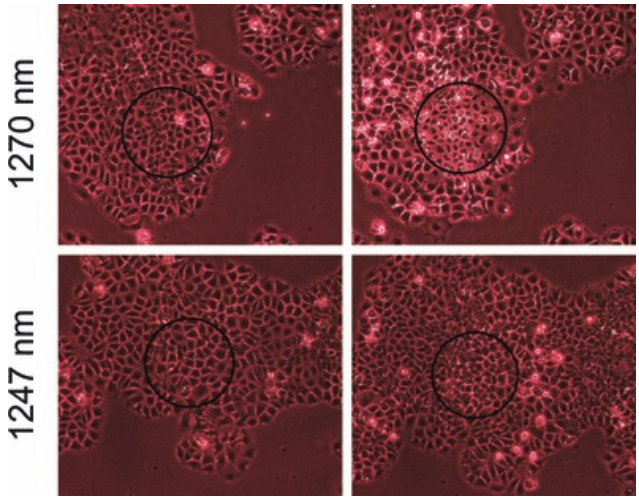

Fig. 2: MCF-7 breast cancer cells irradiated at 1270 nm (top row) and 1247 nm (bottom row) wavelengths. The left and right columns present the cells before and 27 h after laser illumination, accordingly. The area of illumination is indicated by a black circle in all images (∼300 μm). 27 hours after 1270 nm illumination, 100% of cells died in an area with a radius of 200 μm. At the same time, cell death was not observed at 1247 nm [27].

sections of this article, we will show how $^1O_2$ can have such an effect on brain cell activity. In addition, the works [5], [26] show that some other wavelengths of light can be used to excite oxygen molecules. However, in this paper we will consider works on the application of a wavelength of 1267 nm, as the most frequently used and fairly well studied in this area.

III. Cancer Cell Apoptosis Initiation

The specific vulnerability of different cancer cell lines to $^1O_2$ may be used as a potential treatment in some cancers. For example, a number of studies on direct oxygen excitation have demonstrated laser induction of tumour cell death [27], initiation of oxidative stress and destabilisation of cell metabolism [28], the relationship between the dose of laser irradiation and cell death [29], induction of tumour cell-specific apoptosis [30].

F. Anquez et al. showed that irradiation of living human breast cancer cells (MCF-7) at ∼1270 nm wavelength (∼100 W/cm^2, 3 h exposure) can induce cell death (see Fig. 2 [27]). The microscopic images appear to correspond to the morphology of necrotic death associated with a loss of plasma membrane integrity and subsequent osmotic shock. Thermal stress has also been studied, and the authors concluded that cell death was only attributable to the creation of $^1O_2$. It was found, that the obtained cumulative concentration of $^1O_2$, which is necessary to induce cell death, is consistent with the values extracted from conventional PDT.

Works [28], [31], [32] demonstrated that the biological effects caused by laser irradiation are closely related to the excited molecule O_2 which in turn oxidises the biological

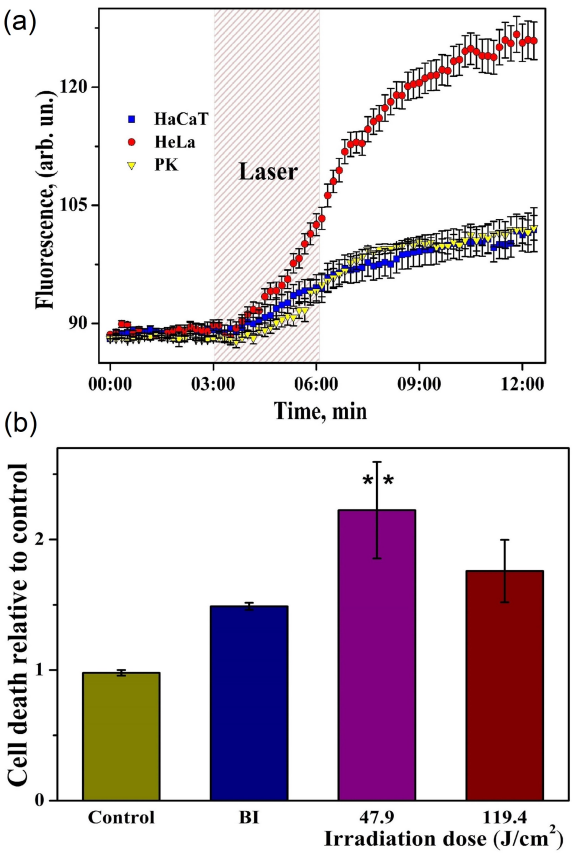

Fig. 3: (a) Changes of 1267 nm-induced DHOE fluorescence in HaCaT cells (blue squares), HeLa cancer cells (red circles), PK (yellow triangles) and (b) HeLa cell death rate. BI is a positive control. Histograms show a Mean±SE (N=3) [28].

substances of cells (proteins, DNA, RNA, phospholipids, etc.) and can finally kill cancer cells. Fig. 3a shows that 1267 nm laser irradiation can trigger $^1O_2$-dependent dihydroxyethidium (DHOE) fluorescence in all cell lines with the most intense impact observed in HeLa cells and without differences between HaCaT and primary keratinocytes (PK). Irradiation was carried out at a dose of 47.7 J/cm^2.

DHOE fluorescence induced by 1267 nm also demonstrated a clear dose dependence without achieving saturation, especially for HeLa cells (doses used: 11.9 J/cm^2, 35.8 J/cm^2, 47.7 J/cm^2, 71.6 J/cm^2 and 119.4 J/cm^2). The authors assume that the high sensitivity of HeLa cells may be due to their malignant activity, which leads to an increased metabolic state and a weakening of the defence system against free radicals [33], [34]. Fig. 3b shows the results of triggering apoptosis in cancer cells. The authors analysed cell death by measuring the activity of lactate dehydrogenase in the extracellular medium. In addition, a positive control of cell death was carried out by treating cells with a PLK1 inhibitor (BI2536).

Kurkov et al. have conducted further experiments using a 1267 nm continuous-wave excitation, where oxidative stress was induced in a cervical carcinoma model (CC-5) in mice [35]. In addition, they demonstrated mitochondrial

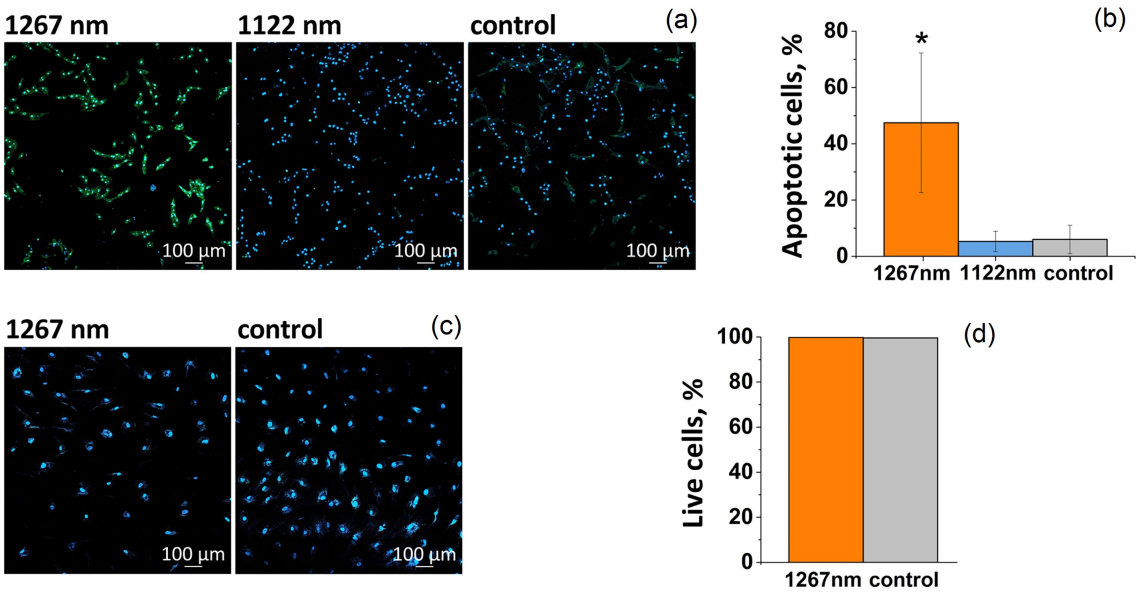

Fig. 4: (a) Confocal images (LSM 900 with Airyscan 2, Carl Zeiss Microscopy GmbH, Germany) of B16 melanoma cells after 1267 nm and 1122 nm illumination and control cells by using NucView 488 and Hoechst 33342; (b) results of statistical processing of cell death; (c) Confocal images of fibroblasts after 1267 nm illumination and control fibroblasts by using NucView 488 and Hoechst 33342; (d) results of statistical processing. Values of $*p<0.001$ were considered significant [30].

oxidative and general cell disruption in the colorectal cancer cell line (HCT-116) and the ovarian epithelium cell line (CHO-K) [29]. The same scientific group has recently demonstrated the possibility of using low doses of radiation to effectively affect cellular metabolism. They have studied cellular damage with respect to the activity of voltage-dependent anion channels (VDAC), oxidative stress level, mitochondrial potential, mitochondrial and nuclear DNA damage in various mammalian cell cultures, including cancer cells, illuminated by low-level laser irradiation (LLLI) at 1267 nm [36]–[39]. LLLI has been shown to cause oxidative stress and apoptosis, and alter mitochondrial functioning even at an energy density of 9.54 J/cm^2. Furthermore, inhibition of VDAC using 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) enhances the registered effects [36].

In work [30] Novikova and colleagues studied human skin fibroblasts and B16 melanoma cell lines. The results confirmed the high selectivity of 1267 nm laser irradiation for generating $^1O_2$, which did not induce the production of other ROS (superoxide anion O_2^-, hydrogen peroxide H_2O_2) or activation of lipid peroxidation. $^1O_2$ did not change the mitochondrial membrane potential ($\Delta\Psi$m) in skin fibroblasts but caused an oscillations in $\Delta\Psi$m and full mitochondrial depolarisation due to opening permeability transition pore (PTP) in B16 melanoma cells. The use of 1267 nm illumination did not change the number of necrotic fibroblasts, but the number of melanoma cells with apoptosis has increased significantly (see Fig. 4). Therefore, $^1O_2$ can induce apoptosis in cancer cells by opening the PTP, but it cannot cause fibroblast death.

More specifically, 1267 nm laser illumination (200 J/cm^2) can activate apoptosis in most B16 melanoma cells (47% of cells: 5448 apoptotic cells of 11,764 cells, Fig. 4a,b). At the same time, less than $<1\%$ of apoptotic cells were observed during illumination of fibroblasts (3 apoptotic cells of 954 cells, Fig. 4c,d). Moreover, the use of a singlet oxygen-free control laser (1122 nm, 200 J/cm^2) also did not show the initiation of cell apoptosis.

IV. Mitochondrial Bioenergetics Stimulation in Brain Cells

As becomes clear from the above, the use of $^1O_2$ in classical PDT or with its direct laser generation in most studies is aimed at killing cancer cells. However, several recent studies have demonstrated that single oxygen can act, for example, as an activator of cellular mitochondrial respiration and thus participate in the stimulation of cell bioenergy. Apparently, mild and constant ROS generation (in particular $^1O_2$) is an essential part of cell signalling [40].

In works [41], [42], the authors investigated the effect of laser-induced $^1O_2$ on the most important oxygen-dependent process - mitochondrial energy metabolism. They found that direct optical generation of $^1O_2$ in neurons and astrocytes induces an increase in $\Delta\Psi$m, activation of NADH- and FADH-dependent respiration and increases the maximum respiration rate in isolated mitochondria. The activation of mitochondrial respiration stimulated the production of adenosine triphosphate (ATP) in these cells. Thus, they found that $^1O_2$ generated by laser irradiation at 1267 nm can act as an activator of mitochondrial respiration and ATP production in brain tissues (Fig. 5).

The authors demonstrated that 71 mW laser irradiation (65.0 J/cm^2 power density) significantly increases intracellular ATP levels. Increasing the laser power to 141 mW (129.2 J/cm^2) and further to 205 mW (187.8 J/cm^2) stimulated more

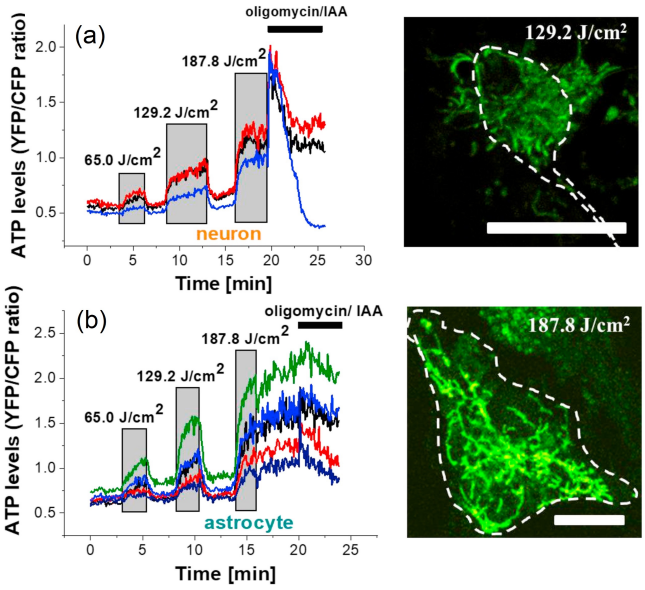

Fig. 5: (a) ATP levels under 1267 nm illumination at different intensities (grey bars) in neuronal cultures using a FRET-based mitochondrial ATP probe [43]. Confocal image of a neuron transfected with the mitochondrial ATP probe and irradiated with 1267 nm. Scale: 50 μm. b) Kinetic changes in mitochondrial ATP of astrocyte while being illuminated with a 1267 nm at different intensities in neuroglial co-cultures. Confocal image of an astrocyte transfected with the mitochondrial ATP probe and irradiated with 1267 nm. Scale: 20 μm [41].

intensive ATP synthesis in neurons (Fig. 5a) and astrocytes (Fig. 5b). ATP production inhibition in these cells using an inhibitor of oxidative phosphorylation of oligomycin and an inhibitor of glycolysis of iodoacetic acid (IAA) caused a significant decrease in ATP levels, proving the effect of $^1O_2$. The authors point out that the main target of the induced effects is mitochondrial cytochrome C oxidase, or Complex IV of the mitochondrial electron transport chain (ETC).

V. Vascular Tone Stimulation

ROS, along with active forms of nitrogen, are known to play a central role in the physiology and pathophysiology of the vascular bed [44], [45]. The response of the vascular bed to the action of ROS is characterised by a multidirectional reaction [46]. It can manifest itself in both the form of vasodilation and the form of vasoconstriction of the vessels. In particular, the $^1O_2$ is able to influence changes in the vascular bed and rheological properties of blood, manifested in stagnation and extravasation of blood, vascular occlusion and shutdown of the vascular network [47]–[49]. The shutdown of the vascular bed of the tumour leads to its hypoxia and, subsequently, to destruction. The totality of these changes is considered the dominant biological response when conducting PDT using PS [49]. However, the generation of $^1O_2$ in the presence of PS does not allow us to draw conclusions about its exceptional effect due to the high direct cytotoxicity of PS

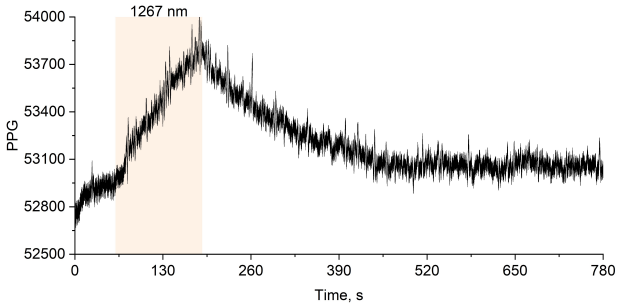

Fig. 6: The photoplethysmography signal from the vascular bed of the rat thigh area under the exposure of 1267 nm irradiation with a dose of 50 J/cm^2 [50]. An increase in the signal corresponds to a decrease in blood content.

and the induction of cellular stress. The possibility of direct excitation of the oxygen molecule by light in the main triplet state and regulation of its production seems promising in the study of the effect of $^1O_2$ on changes in the parameters of the vascular bed.

In a recent work [50], it was shown that laser-induced generation of $^1O_2$ at a 1267 nm wavelength with a 50 J/cm^2 dose can lead to a change in the microvascular bed, namely a decrease in blood content (see Fig. 6) and vasoconstriction and shutdown of microvessels.

The study included continuous recording of images of the vascular network of the femoral and gluteal regions of a rat body with a 250 FPS with laser-induced generation of $^1O_2$. Imaging of the vascular bed was carried out using the method of videocapillaroscopy [51], that is, simultaneous registration of images of backscattered radiation when illuminated by incoherent (525 nm) and coherent (660 nm) light sources. Photoplethysmography (PPG) signals were calculated when processing the obtained series of images. Based on Fourier analysis, maps of the spatial distribution of blood vessels and their blood filling were obtained [52]. Based on analysis of the change in the PPG signal, it was found that direct generation of $^1O_2$ leads to a change in the vascular bed. There is a decrease in blood filling according to the results of PPG, as well as vasoconstriction and stopping of blood flow according to the analysis of processed speckle-images. Here, the authors suggest that the decrease in blood flow may be due to noradrenaline-induced vasoconstriction associated with Ca^{2+}-independent noradrenaline release from the prejunctional site of adrenergic neurotransmission [53].

The possibility of regulating the processes of vasoconstriction and vasodilation of vessels, as well as angiogenesis in the absence of PS using direct optical generation of $^1O_2$ is important for the successful treatment of a whole range of diseases, including diseases of the cardiovascular system, rheumatoid arthritis, atherosclerosis, diabetic angiopathy and retinopathy, psoriasis, oncology, etc.

VI. Stimulation of the Meningeal Lymphatics

The lymphatic vessels (LVs) control immune surveillance and waste elimination within different peripheral tissues and

organs [54]. LVs are not found in the central nervous system (CNS), but are present in the meninges of the brain and spinal cord [55]–[57]. Meningeal lymphatic vessels (MLVs) play an important role in recirculation of dendrite and immune cells, which makes them key players in the control of the brain immune system [58]–[60]. The network of MLVs is also an important pathway for the removal of wastes and toxins from the brain, by participating in the clearance of soluble proteins, as well as in the drainage of the interstitial and cerebral spinal fluids (CSF) that are involved in the mechanisms of homeostasis of the CNS [55], [58]–[64]. The dysfunction of MLVs is involved in the development of numerous neurological diseases, including Alzheimer's [62], [65] and Parkinson's [66] diseases, multiple sclerosis [67], brain tumors [68], [69], stroke [70], and traumatic brain injury [71], [72]. Therefore, MLVs have attracted a lot of therapeutic interest. There is evidence that enhancement of MLV function might be a promising therapeutic target for preventing or delaying neurological diseases [62], [65]. However, currently there are no technologies for modulation of the functions of MLVs.

Photostimulation can be an innovative technology that focusses on the drainage and cleaning functions of MLVs [73], [74]. This approach, also known as low-level laser therapy (LLLT), was first proposed in the 1960s to stimulate hair growth [75] and in the 1970s to heal a wound [76]. Photostimulation is based on the "therapeutic windows" in NIR wavelengths 600–1200 nm. The better tissue penetration properties of NIR light, together with its good efficacy, made it the most popular wavelength range. However, infra-red photostimulation has a significant limitation, such as limited penetration into the brain due to light scattering and heating effects [77]. The light wavelength of 1300 nm has less scattering and can penetrate deeper into the brain [78], [79]. Transcranial photostimulation is considered as a potential new non-pharmacological and non-invasive promising strategy for the prevention or delay of Alzheimer's disease [80]–[84], depression [84], Parkinson's disease [85], stroke [86]–[88], brain trauma [86], [89], [90].

It was recently discovered that $^1O_2$ generation with a wavelength of 1267 nm can be a promising technology for modulation of functions of MLVs [74], [81], [91]–[93]. In particular, 1267 nm laser illumination effectively stimulates the clearance of beta-amyloid (Aβ) from the mouse brain that provides significant improvements in its neurological status [80], [81]. Furthermore, photostimulation has therapeutic effects on intravetricular haemorrhages (IVH), accelerating the evacuation of red blood cells (RBC) from the ventricles, reducing intracranial pressure buildup, improving neurological outcome, and reducing mortality in adult and newborn mice [94]. These findings shed light on our fundamental knowledge about the effects of photostimulation on mature and neonatal brain recovery after IVH and suggest that this approach may be a novel bedside technology, readily applicable, and commercially viable for routine treatment of IVH and other types of brain bleedings.

1267 nm stimulates lymphatic delivery of liposomes to rat glioma, as well as lymphatic clearance of liposomes from the brain [93]. In this pilot study, the authors demonstrate that photostimulation can be a promising technology for modulation of the lymphatic delivery of drugs and nanocarriers to the brain pathology bypassing the blood-brain barrier (BBB). They clearly show that 1267 nm-mediated lymphatic delivery of liposomes with antitumor drugs in the new brain tumour branches might be a breakthrough strategy for the therapy of gliomas.

There are a series of studies devoted to the investigation of the mechanisms responsible for 1267 nm-mediated stimulation of functions of the MLVs [91]–[94]. Low doses (5 and 10 J/cm^2) cause relaxation of mesenteric LVs and increase their permeability to fluorescent macrophages through a decrease in expression of tight junction proteins and transendothelial resistance (Fig. 7). There is a hypothesis that a photostimulated increase in the permeability of the lymphatic endothelium could be the mechanism of transport of macromolecules and cells in narrow MLVs. Increased lymphatic endothelium permeability is the key factor underlying lipids diffusion and macromolecules from tissues to LVs [95], [96]. Indeed, the transport of macromolecules across the LVs is coupled with the flow of water and sensitive to lymph pressure [97]. The inherent permeability of LVs is sufficient to broadcast antigens, passing within the lymph to the lymph nodes [98]. The delivery of soluble antigens, such as FITC-conjugated endogenous proteins and E-GFP is possible due to the permeability of the LVs [99]. This process exposes a large community of immune and dendritic cells, as well as macrophages. However, the mechanisms underlying the lymphatic permeability to macromolecules remain unknown. The possible role of proteins expressed in the lymphatic endothelium, such as the lymphatic vessel endothelial hyaluronan receptor 1 and the chemokine (C-C motif) ligand 21 can be involved in the regulation of migration of immune cells through the lymphatic endothelium [99], [100].

Photomediation of lymphatic transport of different compounds can be explained by the photorelated increase in endothelial NO synthase activity in the lymphatic endothelium [101]. NO acts as a vasodilator by stimulating soluble guanylate cyclase and activating protein kinase G, inducing the opening of calcium-activated potassium channels and the reuptake of Ca^{2+}. The decrease in the intracellular level of Ca^{2+} prevents phosphorylation of myosin light chain kinase that leads to dilation of lymphatic vessels [102]. There are other mechanisms responsible for NO-mediated regulation of lymphatic vasculature relaxation and contractility: 1) the activation of iron-dependent enzymes, including mitochondrial aconitase, an [Fe-S] protein in macrophages [103], 2) inactivation of ribonucleotide reductase [104] and aconitase [105]; the stimulation of synthesis of ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase [106] and protein-sulfhydryl-group nitrosylation [107]. Photostimulation can also cause an increase in lymphatic endothelium permeability through a decrease in the expression of tight junction proteins [92]. Photostimulation has been shown to produce highly pleiotropic biological effects (other than lymphatic engagement) [108], [109]. The well-studied stimulation mechanism is focused on mitochondrial cytochrome c oxidase (CCO), which is responsi-

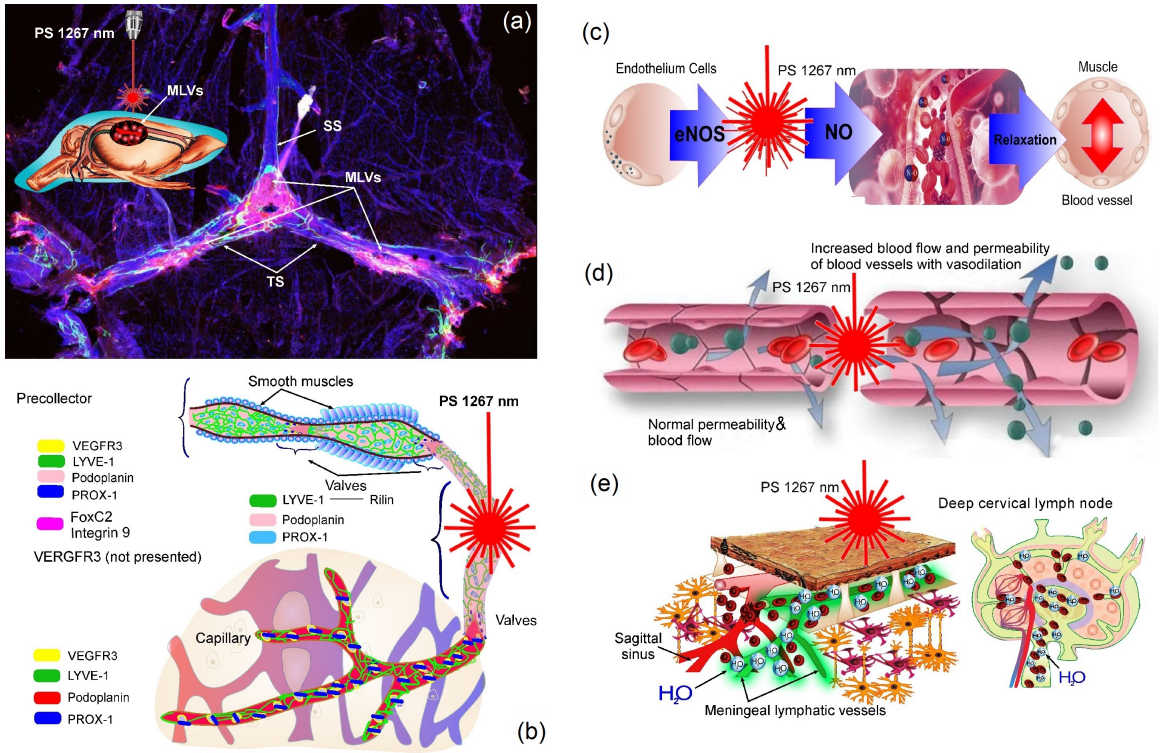

Fig. 7: The mechanisms of 1267 nm stimulation of functions of the MLVs: (a) illustration of effects on the MLVs (pink) along the main vein sinuses (blue), such as the superior sagittal sinus (SSS) and the transverse sinus (TS); (b) schema of structure of the MLVs, including lymphatic capillaries, pre-collectors and collectors with the valves expressing LYVE-1 (the lymphatic endothelium, such as the lymphatic vessel endothelial hyaluronan receptor 1), the CCL-21 (the chemokine (C-C motif) ligand 21), the podoplanin, the PROX1 (the prospero homeobox protein 1), VEGF3 (the vascular endothelial growth factor receptor 3), FOXC2 (the mechanosensitive transcription factor forkhead Box C2), and the Reelin; (c) 1267 nm mediates the NO generation via stimulation of endothelial nitric oxide synthase (eNOS) in the lymphatic endothelium contributing relaxation of the lymphatic vessels and an increase in the permeability of lymphatic walls (d); (e) these mediated modulations of the MLVs are associated with activation of clearance of metabolites and toxins (for example, lymphatic clearance of beta-amyloid (Aβ)) from the brain and their removal into the deep cervical lymph nodes.

ble for the final reduction of oxygen to water using electrons generated from glucose metabolism [110]. Photostimulation causes an increase in NO production, which inhibits the CCO enzyme activity that is accompanied by an increase in mitochondrial membrane potential, proving increased oxygen consumption, increased glucose metabolization, and increased mitochondrial ATP production [90], [111]–[115]. There is evidence that photostimulation stimulates ROS generation in mitochondria. ROS trigger different mitochondrial signaling mechanisms leading to cytoprotective, antioxidant, and anti-apoptotic effects in cells [112].

Sleep is a novel biomarker of the development of Alzheimer's disease [65]. In fact, people with Alzheimer's disease have poor sleep quality and short sleep duration that are associated with increased deposition of Aβ in the brain tissues [116], [117]. Aβ is a metabolic "waste product" of brain tissues and is present in brain fluids [118]. There is experimental and clinical evidence that Aβ clearance increases during sleep due to increased drainage of brain tissues [119]. Interestingly, notice that excessive daytime sleepiness in the elderly is accompanied by increased Aβ accumulation in the brain [120]. Even one night of sleep deprivation provides an increase in the Aβ level in the brain of healthy volunteers [121]. There is a growing body of evidence that disturbance of Aβ clearance from the brain during sleep is a biomarker of Alzheimer's disease, at least in part, via a mechanism of this disease [81], [122], [123]. Based on these facts, recent studies have tested the hypothesis that 1267 nm irradiation during sleep may be more effective for lymphatic stimulation of Aβ clearance from the mouse brain than daytime stimulation. These pilot findings clearly demonstrate that night photostimulation compared to daytime causes greater removal of Aβ from the mouse brain and is more effective as a therapeutic tool to improve the neurological status of mice with Alzheimer's disease [81].

VII. Limitation and Outlook

$^1O_2$ can have a complex effect in the time of induction of cell apoptosis. Moreover, $^1O_2$ production is capable of inducing tumour cell-specific apoptosis. Such a specific vulnerability of cancer cells may be used as a potential treatment in some cancers. Stimulation of nerve cells may be important

in pathological conditions and serious diseases of the brain, such as ischemia or neurodegeneration with a lack of energy production [124]. Although various studies reporting the initiation of apoptosis or optimisation of mitochondrial respiration by laser illumination in various types of cells and tissues have been published, there is still a gap in knowledge and an essential need to identify the exact mechanism by which laser irradiation leads to these effects. Undoubtedly, further efforts should also be directed at reducing the uncertainty in the applied doses, developing systems to detect the concentration of produced $^1O_2$, etc.

Photostimulation of meningeal lymphatics is a pioneering technology for innovative therapy of brain diseases via non-invasive stimulation of clearance of unnecessary metabolites and toxins as well as immune cell communications in the network of the MLVs. However, studies of the mechanisms of such stimulation of MLV functions are still in their infancy, which also requires further detailed investigations in this field. Intriguing are the pilot results of the use of photostimulation of Aβ lymphatic clearance from the brain during sleep that can open a new era in the revolutionary development of smart sleep gadgets aimed at effective therapy of IVH, brain tumours, brain trauma, neuroinflammation and neurodegenerative diseases.

Important and worthy of consideration are the possible temperature effects arising from the interaction of laser radiation with cells and tissues that can affect redox processes [125], [126]. In this regard, it is important to select optimal values of power and radiation doses that exclude or minimise heating. This effect can be achieved, for example, by using ultrashort pulses, which will increase the peak power without significant heating of the tissues. Moreover, in experimental studies it is important to use control lasers having similar heating with sources generating $^1O_2$ to distinguish the observed effects.

This review was devoted to the use of the 1267 nm laser wavelength. However, many studies are being conducted on the use of other wavelengths [5] that can generate $^1O_2$ in different media with different efficiency and thus be also applicable to the tasks of modulating cell bioenergetics. The development of an effective method to generate and use $^1O_2$ can revolutionise the modern practise of treating various diseases. Further research in this area may lead to the creation of new high-tech medical equipment based on the principles of interaction of $^1O_2$ with cells and biological tissues.

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