# Direct generation of $^1O_2$ in living tissues for the treatment of brain diseases

**Oxana Semyachkina-Glushkovskaya***

Molecular oxygen ($O_2$) is the molecule that has created the world at many levels, from the atmosphere to living entities. During the evolution of living organisms, many of them "learned" to use $O_2$ for further oxidation to obtain much more energy. It is therefore not surprising that when living organisms incorporate oxygen into their metabolic system, its reduced chemical forms, known as reactive oxygen species (ROS), assumesimportant regulatory and signaling roles. Currently, oxidation–reduction reactions are central to maintaining life.

Singlet oxygen ($^1O_2$) is one of ROS. For over 20 years, $^1O_2$, is in the spotlight because of its crucial role in the mechanisms of photodynamic therapy of different types of cancer, including brain oncology. Classically, photodynamic therapy is based on the use of photosensitizers that are excited by light and generate a sufficient amount of $^1O_2$ for photo-therapeutic effects. Since photosensitizers specifically accumulate in cancer cells, this provides high concentrations of $^1O_2$ in local tumor area causing cytotoxic effects and leading to cell apoptosis with subsequent suppression of tumor growth.

However, it has turned out that $^1O_2$ can be generated directly in living tissues with light at certain wavelengths, including 1270 nm and 1064 nm.[1-3] The first works in this direction, began in the late 1990s and continued to develop over the course of 20 years, were focused exclusively on cellular studies of the effectiveness of direct $^1O_2$ generation in tumor cells for the purpose of killing them.[1,2] This was largely due to the limited targets whose functions could be modulated using direct generation of $^1O_2$ in the body, where the concentration of $^1O_2$ is obviously lesser than in the isolated cells in *in vitro* experiments. This is due to the significant scattering of radiation energy when passing through the skin, especially through the skull. Indeed, when passing through the scalp, skull, and cerebrospinal fluid (CSF), only 35% of all supplied light energy reaches the surface of the brain. In this sense, there were doubts in the scientific community that direct $^1O_2$ generation would find wide clinical applications.

**Meningeal lymphatic vessels (MLVs) are targets for the physiological effects of $^1O_2$:** The recently rediscovered MLVs have changed the situation and opened up innovative promising prospects for the clinical application of photo-technologies for direct $^1O_2$ generation in the meninges.[4-10] The MLVs are a transparent network located in the dura mater of the brain along the large venous sinuses. These vessels have a mystical history. The MLVs were first described by the Italian anatomist Paulo Mascagni two centuries ago. But, no one else could find them, despite the fact that Mascagni left a detailed description of their anatomy and even wax figures of the human meningeal network, which are still kept in the Museum of Anatomy in Vienna. For two centuries, the dogma prevailed that the brain does not have a lymphatic or immune system. MLVs were rediscovered in 2015 owing to advances in neuroimaging.[11] This discovery opened up a new niche in the treatment of brain diseases, including Alzheimer's disease,[4,7,9] intracranial hemorrhages,[5] and brain cancer.[6] Indeed, recent studies following this event have revealed that MLVs are tunnels for the removal of CSF, metabolites, and toxins from the central nervous system (CNS).[4,5,7]

However, the development of pharmacological methods for modulation of the MLV functions has proven to be a difficult task, as drugs introduced into the blood do not enter MLVs. One of the most promising methods in this direction is the stimulation of lymphoneogenesis by introducing the vascular endothelial growth factor C into the cisterna magna. It should be noted that this is an invasive method, which is limited to widespread use in the clinic and can only be performed for special indications by highly qualified specialists.

The MLVs are located under the skull on the surface of the brain that makes them accessible for photostimulation, because even with the loss of light energy, it is enough to reach the meninges and affect the endothelium of MLVs. The first works in this direction were done using a new generation 1267 nm laser.[4-6,10] The 1270 nm transition has an energy gap of 0.97 eV that pumps a ground state $O_2$ into the first excited singlet state. A pioneering study has shown that the 1267 nm radiation stimulates the MLV functions increasing their contractility and drainage properties via modulation of nitric oxide (NO) regulation of peristaltic functions[5] (**Figure 1A** and **B**). This facilitates lymphatic removal of metabolites and toxins dissolved in CSF from CNS to the cervical lymph nodes, which are the first anatomical station for collection of CSF. Based on these photo-effects, clinically significant results have been obtained in various studies. Indeed, 1267 nm stimulation of lymphatic drainage provides better recovery from intracranial hemorrhages.[5] The 1267 nm activation of brain's drainage contributes to increase in resistance to glioma progression[6] and microglia injury caused by diabetes mellitus.[10] Direct $^1O_2$ generation from this wavelength also stimulates lymphatic clearance of amyloid-β (Aβ), which improves cognitive function in a mouse model of Alzheimer's disease.[4]

**Future perspectives for clinical application of direct $^1O_2$ generation in living tissues:** Light sources emitting around 1270 nm are scarce and expensive, which makes them commercially unattractive. In this regard, light irradiation 1065 nm is much more perspective due to the coincidence of this band with the light emitted by 1270 nm lasers. This band has an energy gap of 1.16 eV that involves a transition of a ground state $O_2$ into the first excited singlet state. Despite the fact that for any biomedical purpose excitation at 1065 nm is equal to that at 1270 nm, there are two main fundamental and practical advantages for 1065 nm. The 1065 nm irradiation has a 10-fold reduction in water absorption as compared to 1270 nm, which allows 1065 nm to save more therapeutic energy. The 1065 nm absorption band coincides with the emission from commercially available lasers (~1064 nm) and light-emitting diodes (LEDs; ~1050 nm). Since LEDs are widely used in clinical practice for photobiomodulation and are recognized by the U.S. Food and Drug Administration as safe technologies, as well as due to their commercially attractive price, they are the most promising for their implementation in clinical practice. Indeed, the first studies in this direction indicate the potential for using LED 1050 nm to effectively remove toxic Aβ from CNS in order to improve cognitive functions of the brain.[7-9]

A unique phenomenon is the fact that sleep significantly increases the therapeutic effects of LED 1050 nm. This is largely due to the natural activation of lymphatic drainage processes in the brain during deep sleep. Phototherapy of brain diseases during sleep is a new direction that is in its infancy.[12] Currently, there are no commercially available devices that simultaneously monitor sleep and deliver photo-radiation with direct $^1O_2$ generation to brain tissue.[12] A prototype of such a device has recently been proposed and tested in preclinical studies as a tool for both effective lymphatic removal of Aβ from CNS and in improving cognitive function in mice with Alzheimer's disease[9] (**Figure 1C**). Sleep-based photobiomodulation technology has also proven effective in facilitating memory formation and new skills in healthy rodents.[8] This device is the world's first technology that allows controlling the MLV functions during sleep by photo-$^1O_2$ generation. It has been adapted for use in humans to remove toxins, including Aβ from CNS and will be tested in clinical trials on patients with Alzheimer's disease in 2025.

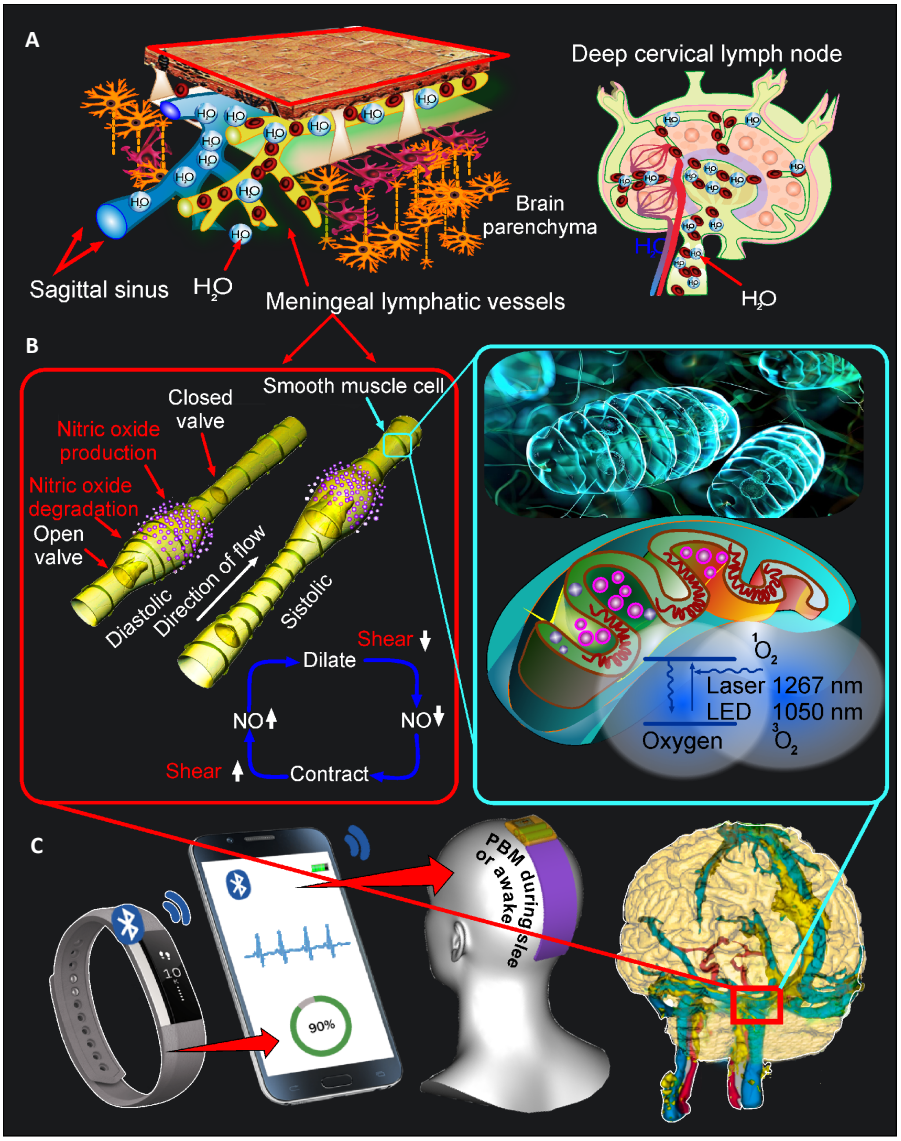


**Figure 1 | A direct photo-$^1O_2$ generation for stimulation of the MLV functions.**
(A) Schematic representation of photo-stimulation of brain's drainage and removal of CSF through MLVs from CNS to the deep cervical lymph nodes. (B) Conceptual scheme illustrating the mechanisms of stimulating photo-effect on MLVs. A laser with a wavelength of 1267 nm or LED with a wavelength of 1050 nm activates the contractility of MLVs by NO- and $^1O_2$-dependent mechanisms. There is a hypothesis based on experimental data that photo-effect on the endothelium of basal MLVs leads to the generation of $^1O_2$ in the mitochondria, which is accompanied by the NO formation, mainly in the valves because 50% of the endothelial NO-synthase is localized there. The release of NO stimulates the dilation of MLVs and increases their permeability, which leads to an increase in their volume due to the influx of fluid into them. Furthermore, NO can react with hydrogen peroxide in the mitochondria, which additionally stimulates the $^1O_2$ generation. At this moment, the upstream valve is open, and the downstream valve is closed. When MLVs are filled, share stress decreases and NO is degraded. Afterward, a subsequent contraction of MLVs is initiated through $Ca^{2+}$ influx both via stretch-, voltage-, or ion-activated channels and from the depot. The contraction of MLVs closes the upstream valves and opens the downstream valves leading to an increase in wall shear stress and the NO production locally, thus starting the cycle again. This way is the peristaltic process in MLVs, which is the basis of their drainage and cleansing functions. (C) Model of PBM technology in sleep and/or wakefulness for stimulation of MLVs. Created with CorelDRAW X7. $^1O_2$: Singlet oxygen; CNS: central nervous system; CSF: cerebrospinal fluid; LED: light-emitting diode; MLV: meningeal lymphatic vessel; NO: nitric oxide; PBM: photobiomodulation.

It should be noted that progress in the development of effective pharmacological methods for the therapy of brain diseases is largely hampered by the presence of the blood–brain barrier, which does not allow 95% of known drugs to enter CNS. This explains the fact that 30% of all known diseases are brain pathologies. In addition, between the development and the appearance on the market of a new pharmacological drug, 10 to 15 years pass.

In this regard, the prospect of developing non-pharmacological and non-invasive technologies based on the use of photo-$^1O_2$ generation for the treatment of brain diseases is promising and commercially profitable with a high chance of rapid implementation in everyday clinical practice.

*This work was supported by grant from the Russian Science Foundation (No. 23-75-30001).*

**Oxana Semyachkina-Glushkovskaya***

Department of Biology, Saratov State University, Saratov, Russian Federation

***Correspondence to:**
Oxana Semyachkina-Glushkovskaya, PhD, glushkovskaya@mail.ru.
https://orcid.org/0000-0001-6753-7513 (Oxana Semyachkina-Glushkovskaya)

**Date of submission:** September 27, 2024
**Date of decision:** November 10, 2024
**Date of acceptance:** November 25, 2024
**Date of web publication:** January 18, 2025

**https://doi.org/10.4103/mgr.MEDGASRES-D-24-00092**
**How to cite this article:** *Semyachkina-Glushkovskaya O. Direct generation of $^1O_2$ in living tissues for the treatment of brain diseases. Med Gas Res. 2025;15(2):208-209.*



## References

1. Blázquez-Castro A. Direct 1O2 optical excitation: A tool for redox biology. *Redox Biol.* 2017;13:39-59.
2. Anquez F, El Yazidi-Belkoura I, Randoux S, Suret P, Courtade E. Cancerous cell death from sensitizer free photoactivation of singlet oxygen. *Photochem Photobiol.* 2012;88:167-174.
3. Sokolovski SG, Zolotovskaya SA, Goltsov A, Pourreyron C, South AP, Rafailov EU. Infrared laser pulse triggers increased singlet oxygen production in tumour cells. *Sci Rep.* 2013;3:3484.
4. Li D, Lin H, Sun S, et al. Photostimulation of lymphatic clearance of β-amyloid from mouse brain: a new strategy for the therapy of Alzheimer's disease. *Front Optoelectron.* 2023;16:45.
5. Li D, Liu S, Yu T, et al. Photostimulation of brain lymphatics in male newborn and adult rodents for therapy of intraventricular hemorrhage. *Nat Commun.* 2023;14:6104.
6. Semyachkina-Glushkovskaya O, Sokolovski S, Fedosov I, et al. Transcranial photosensitizer-free laser treatment of glioblastoma in rat brain. *Int J Mol Sci.* 2023;24:13696.
7. Oxana SG, Alexander S, Inna B, et al. Mechanisms of phototherapy of Alzheimer's disease during sleep and wakefulness: the role of the meningeal lymphatics. *Front Optoelectron.* 2023;16:22.
8. Semyachkina-Glushkovskaya O, Fedosov I, Zaikin A, et al. Technology of the photobiostimulation of the brain's drainage system during sleep for improvement of learning and memory in male mice. *Biomed Opt Express.* 2024;15:44-58.
9. Blokina I, Iluykov E, Myagkov D, et al. Photobiomodulation under electroencephalographic controls of sleep for stimulation of lymphatic removal of toxins from mouse brain. *J Vis Exp.* 2024:e67035.
10. Liu S, Li D, Yu T, Zhu J, Semyachkina-Glushkovskaya O, Zhu D. Transcranial photobiomodulation improves insulin therapy in diabetic microglial reactivity and the brain drainage system. *Commun Biol.* 2023;6:1239.
11. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature.* 2015;523:337-341.
12. Semyachkina-Glushkovskaya O, Fedosov I, Penzel T, et al. Brain waste removal system and sleep: photobiomodulation as an innovative strategy for night therapy of brain diseases. *Int J Mol Sci.* 2023;24:3221.