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Detection of laser-induced singlet oxygen: current approaches and challenges

Irina Novikova[a], Andrey Vinokurov[a], Lybov Eratova[a], Edik Rafailov[b], and Viktor Dremin[a]

[a]Research & Development Center of Biomedical Photonics, Orel State University, Orel, Russia
[b]College of Engineering and Physical Sciences, Aston University, Birmingham, UK

ABSTRACT

This study contains analysis of existing approaches for detecting photosensitizer-free laser-induced singlet oxygen, including their ability to quantitatively measure the relationship between a dose of laser radiation and the amount of singlet oxygen produced.

Keywords: singlet oxygen, direct optical generation, 1267 nm, polarographic method, fluorescence probe (SOSG).

1. INTRODUCTION

Singlet oxygen (SO) is an electronically excited state of triplet oxygen that is less stable than triplet oxygen in the electronic ground state and is produced by photochemical, thermal, chemical, or enzymatic activation of oxygen triplet form.[1] Recently, the mechanism of direct optical excitation of the basic form of oxygen into the singlet state by light at specific wavelengths has been actively studied. Recent research indicates that laser-induced SO may be involved in physiological cellular processes and modulate the cell's redox potential.[1–5] Along with the development of a methodology for the use of SO for therapeutic purposes, an important task is the search for methods to detect SO concentrations. The purpose of this study was to experimentally study existing approaches for the quantitative measurement of SO generation and to analyse their advantages and weaknesses.

2. MATERIALS AND METHODS

The basic triplet state of oxygen has several absorption bands in the infrared and visible regions (optical range between 1300 nm and 390 nm), at which SO can be produced.[6] The sensitivity of the analyzed approaches was studied for laser radiation wavelengths of 1267 nm, 1244 nm and 1122 nm. Laser radiations of 1244 nm and 1122 nm were used as a reference. These wavelengths do not activate the transition of triplet oxygen to the singlet state.[7] During the research, the diameter of the laser beam remained fixed due to the use of a collimator and amounted to 3.4 mm.

The first analysed approach, the polarographic method using the Oxytherm+R respirometer (Hansatech Instruments, UK) was considered. During the experiment, 750 μL of ddH_2O or 5 mM L-histidine solution in ddH_2O was added to the device cell. L-histidine was used as a "chemical trap" in this study. Histidine reacts with SO to form an intermediate product, a trans-annular peroxide, which then rearranges or decomposes into a final oxygenation product.[7] Thus, it make possible of polarographic measurements of a dissolved oxygen concentration decrease. During the experiment, the temperature in the measuring chamber was 26 °C. The laser radiation was delivered through the glass wall of the measuring chamber.

The second analysed approach, fluorescence intensity measurements with Singlet Oxygen Sensor Green (SOSG) (Invitrogen, USA) was used. This probe initially has a weak blue fluorescence, but transforms into a form with intense green fluorescence after selective interaction with SO. The concentration of SOSG in the measuring chamber during the experiments was 5 μM. Measurements were made using a Zeiss LSM 900 microscope (Carl Zeiss AG, Germany) with a 10× objective. To excite the fluorescence of the indicator, a laser with

Send correspondence to Viktor Dremin
E-mail: viktor.dremin@bmecenter.ru

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a wavelength of 488 nm and a power of 0.1% of the maximum was used. Delivery of laser radiation during the experiments was carried out from the opposite side of the microscope objective.

The protocol includes a 3-minute recording of the basal fluorescence intensity with subsequent SO generation by laser radiation (the duration depended on the chosen dose) and a 6-minute recording of the signal after the laser exposure. The sensitivity to the SO formation when exposed to the selected one (200 J/cm^2) and various doses (50 J/cm^2, 100 J/cm^2, 150 J/cm^2, 200 J/cm^2, 250 J/cm^2) of laser radiation at a fixed power value was analysed. To reduce the thermal effect of the absorbed radiation on the measurement results, the laser power was 50 mW.[8]

The results of the experimental data were presented as a relative increase in the intensity of SOSG fluorescence compared to the initial level taken as 100%.

3. RESULTS AND DISCUSSION

Fig. 1 shows the results of the measurements using the polarographic method using the Oxytherm+R respirometer. As can be seen from the results, laser exposure with a wavelength of 1267 nm leads to a more significant decrease in the dissolved oxygen level in the measuring chamber compared to experiments with control lasers at 1244 nm and 1122 nm (Fig. 1a). However, this decrease is more pronounced in the case of L-histidine solution (Fig. 1a,b).

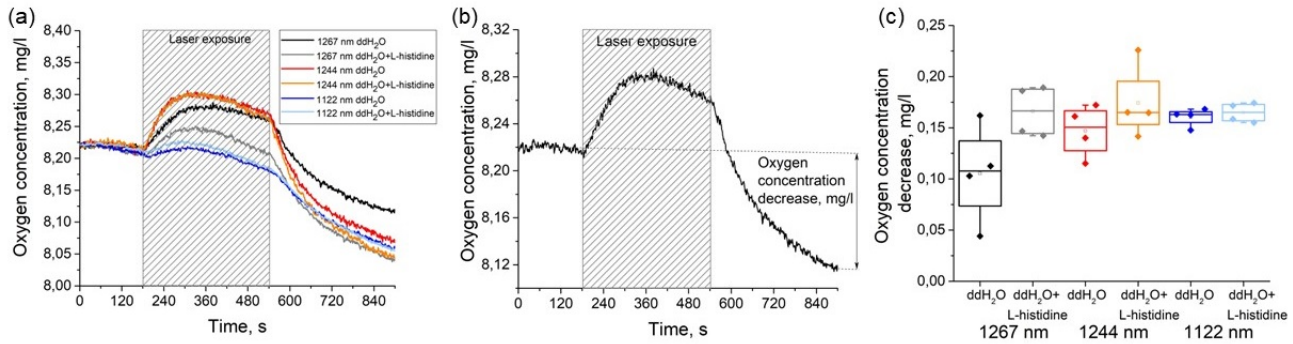

Figure 1. Results of application of the polarographic method with Oxytherm+R respirometer at dose of laser - 200 J/cm^2, power - 50 mW, 4 repetitions for each wavelength in μL of ddH_2O or 5 mM L-histidine solution (24 measurements)

However, despite the shown differences between the main and reference lasers, this approach is characterised by insufficient sensitivity to the detection of various doses of generated SO. Also, the high sensitivity of the Clarke electrode to temperature fluctuations, despite the temperature stabilisation in the measuring chamber, does not allow one to analyse the change in the signal at the moment of laser exposure. Therefore, only an indirect estimate can be made after calculating the final decrease in oxygen concentration in comparison with its initial level before exposure.

Fig. 2 shows the results of SOSG fluorescence measurements. As can be seen from the data presented in Fig. 2a,b, at the moment of exposure to laser with wavelength of 1267 nm, an increase in SOSG fluorescence is observed, which indicates SO production. A similar growth of the signal occurs under laser radiation at a wavelength of 1244 nm. This result may indicate the production of SO and the incorrect use of a laser with a wavelength of 1244 nm as a reference, in contrast to a laser with a wavelength of 1122 nm. This approach is also characterised by the sensitivity to differences in the amount of SO generated by different doses of laser radiation (Fig. 2c).

4. CONCLUSION

In this work, we presented the results of an analysis of two approaches for the detection of SO generation by using instrumental methods with a chemical trap or a SO-selective fluorescent probe. These approaches are characterised by different sensitivity to SO detection. However, these methods allow one to realise only an indirect

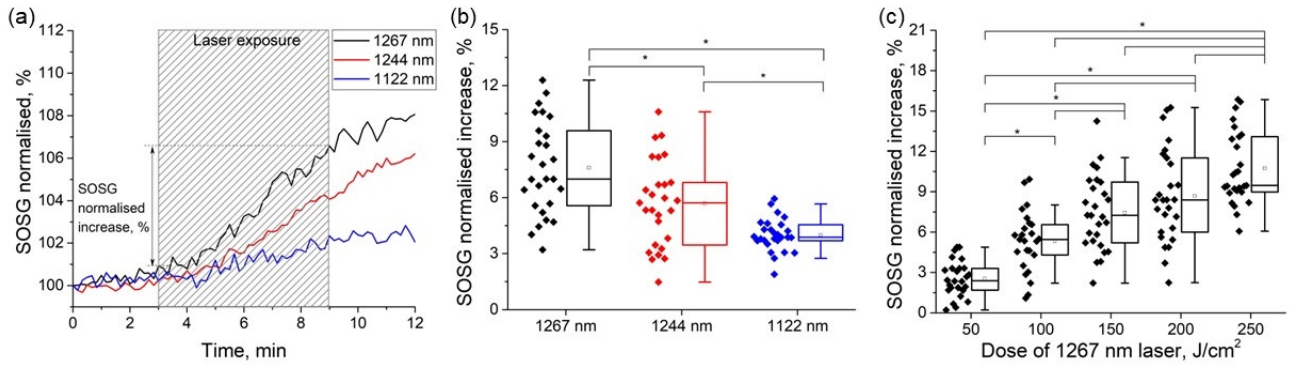

Figure 2. Results of application of method with SOSG fluorescence intensity measurements (a-b) at dose of laser - 200 J/cm^2, power - 50 mW (3 repetitions for each wavelength (12 measurements), 8 regions of interest in each repetition); (c) at doses of laser - 50 J/cm^2, 100 J/cm^2, 150 J/cm^2, 200 J/cm^2, 250 J/cm^2, power - 50 mW (3 repetitions for each wavelength (30 measurements), 8 regions of interest in each repetition)

estimation and cannot provide SO-generation control during the laser action. In the case of the polarographic method only generated in solution or diffused from the tissue SO can be measured. But the last seems impossible due to the low SO lifetime. The use of SOSG is significantly limited by its poor penetrating ability through the cell membrane. Therefore, the use of chemical traps or specialised fluorescent probes is suitable only for a narrow type of tasks.

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