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Effect of Oxygen Concentration on Singlet Oxygen Signal and Treatment Outcome Measured by Multispectral Singlet Oxygen Luminescent Dosimetry (MSOLD) In Tumor-Bearing Mice

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ABSTRACT

Singlet oxygen (SO) is the primary cytotoxic species in photodynamic therapy (PDT), but real-time in vivo measurement remains difficult. This study evaluated multispectral singlet oxygen luminescence dosimetry (MSOLD) for monitoring SO during benzoporphyrin derivative–mediated PDT.

MSOLD was tested in water and solid phantoms and then applied in RIF tumor–bearing C3H mice during oxygen modulation with medical air and carbogen. Clear SO signals were detected, increasing during carbogen breathing and decreasing during medical-air breathing. BPD-mediated PDT was subsequently delivered at different treatment doses, and therapeutic outcome was correlated with the measured PDT dose.

These results support MSOLD as a feasible real-time dosimetry and treatment-monitoring tool for BPD-PDT.

INTRODUCTION

Photodynamic therapy (PDT) is a minimally invasive treatment that combines a photosensitizer, light of an appropriate wavelength, and molecular oxygen to generate cytotoxic reactive oxygen species. Among these species, singlet oxygen (SO) is considered the primary mediator of tumor-cell damage. PDT efficacy therefore depends on several dynamic factors, including photosensitizer concentration, delivered light dose, and tissue oxygen availability.

Benzoporphyrin derivative (BPD) is a clinically relevant photosensitizer with strong absorption near 690 nm, allowing deeper light penetration into tissue. After administration, BPD accumulates in tumor tissue and, upon light activation, transfers energy to molecular oxygen to produce SO. However, treatment response can vary because of heterogeneous drug uptake, oxygen depletion, and differences in tissue optical properties.

Direct monitoring of SO may therefore provide a more biologically relevant measure of PDT dose than light dose alone. In this study, multispectral singlet oxygen luminescence dosimetry (MSOLD) was evaluated for real-time monitoring of SO during BPD-mediated PDT and for investigating its relationship with tissue oxygenation and treatment outcome.[1-2]

METHODS AND MATERIALS

MSOLD measurements were first performed in a water-based phantom and a water-based Agarose solid phantom to demonstrate detection of the singlet oxygen SO luminescence signal under controlled conditions.

In vivo MSOLD measurements were then conducted in tumor-bearing mice during breathing of medical air and carbogen. The resulting changes in SO signal were used to confirm that MSOLD was sensitive to variations in tumor oxygenation.

Finally, BPD-mediated PDT was performed using a fixed BPD concentration and a fluence rate of 100 mW/cm². Different treatment times were used to deliver different light doses, and treatment outcome was evaluated in relation to the measured PDT dose.

RESULTS

Figure1 demonstrates MSOLD performance in controlled phantom experiments. Clear singlet oxygen luminescence signals were detected in both phantoms, and spectral decomposition separated the singlet oxygen component from photosensitizer luminescence and direct light. The measured singlet oxygen signal increased approximately linearly with BPD concentration in both phantoms, confirming the sensitivity and quantitative response of the MSOLD system.

Figure2 shows the in vivo application of MSOLD during BPD-mediated PDT in RIF tumor-bearing mice. MSOLD successfully resolved the singlet oxygen signal during treatment, while

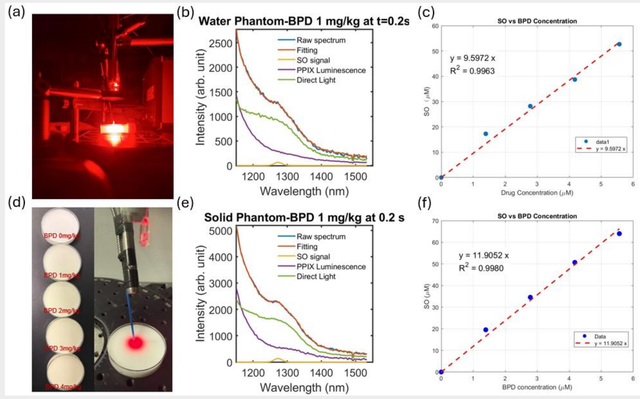

Figure 1. MSOLD in-vitro experimental setup and signal characterization. (a) MSOLD system configuration with a water phantom. (b) SVD fitting of MSOLD signals obtained from the water phantom. (c) MSOLD-measured SO signal at different drug concentrations in the water phantom. (d) MSOLD system configuration with a solid phantom. (e) SVD fitting of MSOLD signals obtained from the solid phantom. (f) MSOLD-measured SO signal at different drug concentrations in the solid phantom.

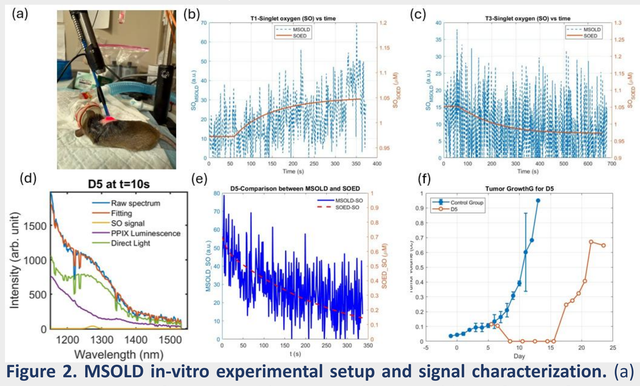

Figure 2. MSOLD in-vitro experimental setup and signal characterization. (a) MSOLD system configuration with a water phantom. (b) SVD fitting of MSOLD signals obtained from the water phantom. (c) MSOLD-measured SO signal at different drug concentrations in the water phantom. (d) MSOLD system configuration with a solid phantom. (e) SVD fitting of MSOLD signals obtained from the solid phantom. (f) MSOLD-measured SO signal at different drug concentrations in the solid phantom.

simultaneous oxygen measurements demonstrated changes in tumor oxygenation. The singlet oxygen signal increased during carbogen breathing and decreased during medical-air breathing, confirming its dependence on oxygen availability. Tumor growth monitoring was then used to evaluate treatment response and relate the measured PDT dose to therapeutic outcome.

Table 1. Summary of the treatment Schemes, treatment time, PDT dose, mean calculated reacted reactive oxygen concentration $[ROS]_{rx}$, and local control rate (LCR)

Groups	# of Mice	Treatment Time (s)	Planned PDT dose (uMJ/cm²)	$[ROS]_{rx}$ (mM)	LCR
Control	8	0	0	0	0
1	4	300	42	0.338±0.088	0
2	5	600	83	0.575±0.165	0
3	7	900	125	0.62±0.065	0
4	7	1260	175	0.806±0.14	0.286±0.488
5	6	1620	225	1.343±0.306	0.333±0.516
6	7	1800	250	1.631±0.509	0.429±0.535
7	4	1980	275	1.718±0.561	0.750±0.500

The Kaplan–Meier curves show improved and more sustained local tumor control with increasing PDT dose, with the highest-dose groups maintaining the greatest control over the 90-day follow-up as shown in Figure 3(a). When the 90-day local control rate was plotted against

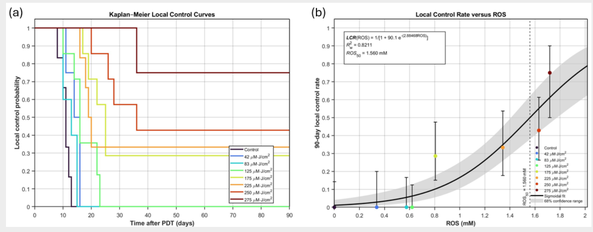

Figure 3. PDT-induced local tumor control. (a) Kaplan–Meier local control curves for the control and PDT groups. (b) Ninety-day local control rate versus ROS with a sigmoidal fit.

measured ROS, the data followed a sigmoidal dose–response relationship, indicating a sharp increase in treatment efficacy once sufficient ROS was generated. The fitted curve gave an estimated ROS_{50} of 1.56 mM, representing the ROS level associated with a 50% local control rate as shown in Figure 3(b).

DISCUSSION

Phantom experiments demonstrated that MSOLD can detect SO, with the measured signal increasing linearly with photosensitizer concentration in both liquid and water-based solid phantoms. The in vivo study further supported MSOLD as a real-time PDT dosimetry method, as the SO signal increased with oxygen concentration and decreased under oxygen-limited conditions. In the BPD-mediated mouse study, higher cumulative SO was associated with improved long-term tumor control, with a median effective value of approximately 1.56 mM SO for achieving 50% local control at 90 days. However, the fitted response was affected by the small group sizes, variability in tumor oxygenation, and uncertainty in ROS measurements. Higher PDT doses should be evaluated to determine whether complete and durable tumor control can be achieved.

CONCLUSIONS

MSOLD demonstrated reliable SO detection in both phantom and in vivo studies and showed sensitivity to photosensitizer and oxygen concentrations. In the BPD-mediated mouse study, cumulative SO was associated with long-term tumor control, with an estimated ROS_{50} of 1.56 mM at 90 days. These findings support MSOLD as a promising real-time PDT dosimetry method, although larger cohorts and higher PDT doses are needed to validate the response relationship and determine conditions required for complete tumor cure.

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