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Detection of singlet oxygen production for PDT treatments both in vitro and in vivo using a diode laser-based singlet oxygen monitor

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ABSTRACT

Photodynamic therapy (PDT) is a promising cancer treatment. PDT uses photosensitizers that are selectively retained in malignant tumors. When tumors, pretreated with the photosensitizer, are irradiated with visible light, a photochemical reaction occurs and tumor cells are destroyed. Oxygen molecules in their metastable singlet delta state, O2(1∆), are believed to be the species that destroys cancerous cells during PDT. Physical Sciences Inc. (PSI) has developed a fiber optic-coupled, diode laser-based diagnostic for singlet molecular oxygen produced during PDT. This portable prototype system detects the singlet oxygen emission near 1.27 µm. We have obtained signals from singlet oxygen during and following pulsed laser excitation and demonstrated excellent sensitivity with this system. Both in vitro and in vivo studies have been completed, and we have detected the production of singlet oxygen during PDT treatments with both animal and human subjects. Effects of photobleaching have also been observed. These results are promising for the development of the sensor as a real-time dosimeter for PDT.

Keywords: Photodynamic therapy, singlet molecular oxygen, diode laser, real-time monitor

1. INTRODUCTION

Photochemotherapy of cancer is often called photodynamic therapy (PDT).1 In PDT, a photosensitizer (PS) is injected to a cancer patient, and this agent is preferentially retained in tumor cells. The agent is irradiated with visible to near infrared light. The wavelength of the light has to match the absorption of the photosensitizer. The excited PS can transfer its energy to oxygen molecules to generate singlet oxygen (O2(a1∆)), and the singlet oxygen can produce cell necrosis. FDA approval has been granted for treatment of esophageal and certain lung cancers. PDT is being used in clinical trials for bladder, brain, skin and other cancers.2-6 PDT is also being applied to important areas outside of cancer treatment including age related macular degeneration and actinic keratosis, a pre-cancerous skin condition.

There is considerable evidence that O2(a1∆) is the active species in cancer cell or endothelial cell necrosis. Despite the general acceptance of this role of O2(a1∆) in PDT there have been limited demonstrations of its importance in vivo.7 If O2(a1∆) is indeed the critical species that determines PDT efficacy, a device that is conducive to on-line measurement of O2(a1∆) in vivo could, in principle, provide the critical parameter in PDT dosimetry and the potential of individualized therapeutic design.

PSI’s diode laser approach is summarized in Figure 1.9 The diode laser operates in pulsed mode with a peak power < 300 mW. It generates prompt dye fluorescence. Since O2(a1∆) lifetime is longer than dye fluorescence, we can detect the singlet oxygen signal after the diode laser turns off. We detect the singlet oxygen fluorescence with optical filtering of O2(a1∆) emission spectral region.

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In a series of papers Wilson et al.\textsuperscript{7,8,10,11} have reported a singlet oxygen detector based upon a similar PMT and pulsed excitation with a Q-switched, frequency doubled, Nd:YAG laser. Our method is distinct from Wilson’s in several ways. They use a frequency-doubled Q-switched Nd:YAG laser or an optical parametric oscillator to detect PDT-produced singlet oxygen emission both \textit{in vitro} and \textit{in vivo}. Note that unlike Q-switched Nd:YAG lasers (pulselengths \textapproximately 10 ns), our relatively long (~ 5 µs) diode laser pulses do not produce significant energy compression. While the diode laser is on, its peak power is essentially equal to that when operating CW. Consequently, our peak powers are much lower. For example for a 300 mW diode laser, pulses of 2 µs duration contain only 0.6 µJ and each pulse has a peak power of 300 mW and can cause no tissue damage. Wilson and co-workers also use a freespace laser beam and macroscopic lenses for beam focus and singlet oxygen emission collection in contrast to our fiber coupled system.

Previously, we reported the results of our initial investigation to develop a sensitive, diode laser-based monitor for singlet oxygen produced by light treatment.\textsuperscript{9,12} Using a pulsed diode laser and photon counting methods, we demonstrated singlet oxygen detection during PDT of rat prostate tumor cells \textit{in vitro} as well as in solution phase. In this paper, we discuss our progress on the system performance for singlet oxygen monitoring. We report the first time measurements of \(O_2(a^1\Delta)\) in tumor-loaded rats and healthy human skin.

### 2. EXPERIMENTS

Figure 2 shows the current prototype PSI singlet oxygen monitor. This is more compact and easy to operate compared to our previous experimental setup.\textsuperscript{9} All components are in one equipment rack including: 1) diode laser module; 2) PMT detector; 3) data acquisition system with photon counting board. The light delivery and collection are fiber coupled, so there are no external optics that require alignment. Singlet oxygen has very weak emission, and the spectral region of singlet oxygen slightly overlaps with photosensitizer fluorescence. To differentiate the background emission such as photosensitizer fluorescence and autofluorescence (albeit weak) from recorded \(O_2(a^1\Delta)\) signal, we used three-optical filter system. In front of the PMT, we integrated a filter slider that contains three optical filters (peak emissions at 1.22, 1.27, and 1.315 µm). This filter slider is for effective background subtraction, especially for \textit{in vivo} studies. We corrected each spectrum with a background spectrum.

The fast photon counting board can handle up to a 20 kHz operation rate. We wrote custom software that controls: 1) the diode laser module controlled through a National Instruments GPIB card; and 2) a graphic user interface (GUI) to control all parameters of photon counting system and to display the data acquisition in real time from the fast photon counting system.

Our device measures directly the singlet oxygen dose which is proportional to the product of the PS concentration, the ground state oxygen concentration and the light intensity within the tissue being irradiated. Thus, we can define the dose as

\[
\int [PS(t)] \cdot [O_2] \Phi(t) \, dt
\]

where \([PS(t)]\) is the photosensitizer concentration, \([O_2(t)]\) is the ground state oxygen concentration and \(\Phi(t)\) is the light irradiance.
In tumor cell experiments at PSI, we used a rat prostate cancer cell line incubated with the photosensitive dye chlorin e6 (Cl e6) about 4 hours. Before PDT experiments, the medium was removed and the cells were washed with phosphate buffer solution. Therefore, Cl e6 dye that was not taken into the tumor cell was removed. The observed singlet oxygen signal was from intracellular singlet oxygen. In addition, we confirmed that the singlet oxygen signal was from the tumor cells, not from the suspension, from experiments of cell pallets.

We measured O$_2$(a$^1\Delta$) production during PDT treatments at the Wellman Photomedicine Research Center at MGH. Two in-vivo studies were performed. First, a rat prostate cancer model was studied with several PDT photosensitizers such as Cl e6, ALA-induced PpIX, and BPD. Second, we extended our study to healthy human subjects using topical ALA-induced PpIX case. Below we provide a short summary of these results. Details will be provided elsewhere.

3. RESULTS

3.1 In vitro studies
The suspension of tumor cells was put into a glass bottle and the solution was mixed with a magnetic stirrer. Each spectrum was corrected by the background fluorescence of the cell suspension without dye, such as autofluorescence from biomolecules or fluorescence from optical components. With the initial dye concentrations of 100 $\mu$M and 10 $\mu$M, we readily observed the temporal evolution of the O$_2$(a$^1\Delta$) signal in a rat prostate tumor cell line, as shown in Figure 3.

![Figure 3: Temporal evolution of O$_2$(a$^1\Delta$) emission in a rat prostate tumor cell line using Cl e6.](image)

3.2 In vivo studies
In the tumor-loaded rat study, we monitored O$_2$(a$^1\Delta$) signal intermittently during the PDT treatment. The treatment laser was shut off during these measurements. We investigated the relationship between PDT, O$_2$(a$^1\Delta$) production and local tumor growth outcome. Figure 4 shows the typical decay profiles of singlet oxygen produced from a tumor implanted
on a rat using ALA as the PS: Figure 4a) before ALA injection and Figure 4b) after PDT treatment with ALA. The lines shown are fits to the temporal evolution data. To maximize the sensitivity of the sensor, we corrected the singlet oxygen emission by subtracting background signals due to photosensitizer fluorescence and autofluorescence.

![Graphs showing singlet oxygen decay](image)

Figure 4: The typical decay profile of singlet oxygen produced in the tumor burdened rats: (a) before ALA injection, (b) 100 mg/kg body weight ALA and 50 J irradiation.

Following the PDT treatment with different photosensitizer dosages and light dosages, the tumor size was measured each day for 14 days. The control group with no photosensitizer and/or no light treatment showed steady tumor increase.

In contrast, for PDT treated tumors that produced measurable singlet oxygen there was a correlation between the tumor regression and singlet oxygen produced. For example, for two different animals each treated with the same amount of PS, one produced a small singlet oxygen signal and the other produced nearly six times as much singlet oxygen. The tumor on the first animal showed persistent growth, while the tumor on the second regressed by a factor of ten in the first two days after treatment, before regrowth began. Details on these results will be reported elsewhere.

We also conducted a pilot study with 15 healthy human subjects using ALA-induced PpIX and were able to detect singlet oxygen. To our knowledge this is the first observation of \( \text{O}_2(\alpha^1\Delta) \) production on human skin with PDT treatment. The relationship between \( \text{O}_2(\alpha^1\Delta) \) production and photosensitizer fluorescence was also investigated. We observed that the \( \text{O}_2(\alpha^1\Delta) \) production maximized after about 10 J of treatment light. In contrast, the photosensitizer steadily decreased from its initial value prior to light treatment. The results may indicate increased oxygenation during PDT even during photosensitizer photobleaching.

4. SUMMARY

Using our diode laser based system, we have observed \( \text{O}_2(\alpha^1\Delta) \) produced from PDT treated tumors on live rats and through follow-up studies, observed tumor shrinkage that correlated with the amount of \( \text{O}_2(\alpha^1\Delta) \) produced. These measurements are the first of their kind with a fiber optic coupled device. We also observed the first measurements of \( \text{O}_2(\alpha^1\Delta) \) production in human subjects. We plan to improve the singlet oxygen monitor sensitivity for more extensive in vivo studies.

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