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Comparison of pulse and super pulse radiation modes' singlet oxygen production effect in antimicrobial photodynamic therapy (AmPDT)

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ABSTRACT

Photodynamic Therapy (PDT) is a treatment method in which a target region is irradiated with a light source of an appropriate wavelength to activate an introduced photosensitizer to ideally ablate the target by creation of highly toxic singlet oxygen. Due to the increment of antibiotic resistant bacteria, PDT has also become a salient method for infection treatments. The amount and the location of singlet oxygen gives information about the effectiveness of PDT. The quantitative evolution of singlet oxygen is a gold standard for the real time monitoring of the treatment efficiency during PDT.

In the proposed study, the effect of radiation modes on PDT is investigated with singlet oxygen explicit dosimetry (SOED) and singlet oxygen luminescence dosimetry (SOLD) methods. For this purpose, super pulse and pulse radiation modes are applied for antimicrobial PDT (AmPDT). Five in vitro experiments were carried out to investigate the effect of radiation mode. According to the achieved results, super pulse mode provides 3–10 % more singlet oxygen concentration and 2–5 % more bacteria (Staphylococcus Aureus) death (necrosis and apoptosis) than pulse mode. Furthermore, radiation mode effect on instantaneous and cumulative singlet oxygen concentration is considered in the experiments. It is demonstrated that the singlet oxygen concertation measured by SOED and SOLD methods are coherent. Thus, the SOED method can be used for real-time singlet oxygen measurements during PDT.

1. Introduction

Photodynamic therapy (PDT) is an emerging treatment option that can be utilized in a wide application area such as cancer, rheumatoid arthritis, and infection. The successful PDT treatment can be maintained with the presence of three key components: light, photosensitizer and oxygen. Singlet oxygen concentration is an indicator of PDT dosimetry efficiency. PDT dosimetry calculations are made by setting the doses of photosensitizer and light. This is often inadequate due to the differences in nature of target environment [1,2]. In addition, optical properties and oxygenation of the target environment can affect the treatment outcome [3,4].

In order to achieve an efficient PDT dosimetry technique, various methods including implicit dosimetry, explicit dosimetry, and the biophysical/biological response monitoring have been proposed in the literature [9–11]. In the computational model of SOED, the best-defined amount of biophysical dosimetry is the light dose absorbed by the photosensitizer that is proportional to the integral time of the local light photosensitizer concentration and optical power. Under well-oxygenated conditions, PDT dose provides a valuable information about the treatment process. However, for poorly oxygenated targets, the high fluence rate can create even more severe hypoxia during illumination [4–6]. Therefore, to characterize the outcome of PDT treatment, oxygen consumption of bacteria or cancer cells must be taken into account. In recent studies, singlet oxygen luminescence-based dosimetry models considering the oxygenation properties were proposed by the researchers. SOLD method, for example, is used to detect the singlet oxygen at 1270 nm wavelength [7,12–14]. Also, there are various detection systems to monitorize the singlet oxygen illumination such as large monochromatic-based instruments including infrared photomultiplier tube (IR PMT) and Indium Gallium Arsenic single-photon avalanche detectors (InGaAs SPADs) [15–19]. These systems could be used as a potential PDT dosimetry tool or to obtain information about physiological parameters that change during the treatment, such as oxygen partial pressure of the target area [6–8]. Although IR PMT and InGaAs SPADs based systems are proven to be useful for experimental setups, their dependency on the environmental parameters and costliness make them inaccessible for the clinical usage.

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According to the discussions given above, singlet oxygen concentration can be used as a dosimetry measure with SOED and SOLD techniques for a PDT treatment. The direct measurement of singlet oxygen by its near-infrared luminescence emission is difficult because of the signal weakness and short lifetime of singlet oxygen (~ 10 ns - 15 us) [20–22]. As a result of this, PDT dosimeter systems hard to implement especially for infection dosimetry due to the nature of bacteria. If proper techniques can be attained, the PDT will be a powerful choice for disinfecting the open wounds, water, foods, infected tissues, prosthetics and implants.

Staphylococcus Aureus (SA), which is drug-resistant and dangerous bacteria, cause serious infections for the diabetic, prosthetic-implanted and wounded patients. Antibiotics are currently the most common drugs used for infection treatments, but are not adequate to prevent antibiotic-resistant bacteria such as SA [23–25]. According to the literature review, antimicrobial photodynamic therapy (AmPDT) can be considered as a bactericidal and effective against SA [26–29]. However, to achieve a successful AmPDT the key components needs to be monitored. The light dosimetry, PS dosimetry, oxygen consumption of bacteria and re-oxygenation are the important aspects of AmPDT.

There are a lot of photosensitizer having a special bandwidth and peak wavelength. An ideal photosensitizer has a high excitation coefficient in the range of 600 − 800 nm. The wavelengths below 600 nm are strongly absorbed by biological molecules and the photons above 800 nm are not energetic enough to excite the photosensitizer [30]. Within 600 − 800 nm wavelength, the light penetration on the water-based solutions and tissues is much deeper and so singlet oxygen generation during PDT is much [31,32]. The photosensitizers that have near infrared (NIR) peak absorption are more suitable for disinfection applications. Among them, methylene blue has a maximum absorption peak between 664 and 631 nm [33]. After specifying PS, a proper light source must be chosen to deliver peak excitation wavelength of the PS in the range of desired optic power level. There are several light sources used to deliver the peak absorption point of the PS. The light sources are chosen by considering its optic power stability and bandwidth. Lasers and LEDs have been used as a light source for AmPDT applications[34–39]. The latest researches tent to use LED light sources due to their lower costs and valuable performances in the bacterial field [40–43].

In this study, singlet oxygen generation in bacteria cultured methylene blue solutions was investigated for pulse and super pulse radiation modes. For this purpose, singlet oxygen production levels of the solutions having different methylene blue proportions were experimented under pulse and super pulse radiation modes. SOED calculations and SOLD measurements with avalanche photodiode based singlet oxygen detection system (SODS) was made. AmPDT adapted SOED and SOLD models were compared with each other for each experiment and the efficiency of the super pulse mode was investigated by SOLD measurements.

One of the targets of the presented study was to evaluate the bactericidal effect of AmPDT via singlet oxygen luminescence by using computational and measurement-based dosimetry methods. Pulse and super pulse radiation modes of the LED light source were compared with regard to their singlet oxygen production concentration during AmPDT. Five main experiments were performed for the proposed study. In these experiments, different concentrations of methylene blue (1% and 5%) were used with LED light sources having 10 J/cm^2 energy density and 660 ± 10 nm wavelength under pulse and super pulse modes. For each experiment, singlet oxygen instantaneous and cumulative concentrations were obtained with SOED and SOLD methods. According to the results, both radiation modes can be applied to AmPDT applications. Super pulse mode provides relatively high SA mortality in vitro by increasing the singlet oxygen concentration percentage about 3–10 %. Besides, it is proven that the singlet oxygen concentration measured by SOED and SOLD methods were very close to each other. So, SOED systems can be preferred to overcome the complexity and expensiveness of the SOLD based systems. However, for real time monitoring SOLD based system must be used. For more accurate dosimetry method SOED aided SOLD methods must be developed.

2. Materials and methods

In this section, the SOED was adapted to AmPDT application and the experimental procedure was explained. The required parameters were modified to calculate the singlet oxygen production level via SOED and calculated singlet oxygen concentrations were compared with SOLD model measurements.

2.1. Bacterial strain and culture condition

The bacterial strain used in this study was SA. Cells were cultured in sheep blood agar at 37 °C and grown for 24-h. For the experiments, colonies were collected with the aid of a calibrated loop of 100 μL and inoculated into distilled water. Bacteria were plated in a petri dish culture plate [50]. After this inoculation, each concentration of the photosensitizer was added and irradiated following the experimental protocol.

2.2. Singlet oxygen generation for pulse and super pulse modes

Photosensitizers can be divided into two categories as type I and type II. Most photosensitizers used in the clinical applications are of type II which produce singlet oxygen as the main photocytotoxic agent for events causing necrosis and/or therapeutic effect. In type II process, energy transfer from an excited photosensitizer to triplet oxygen produces singlet oxygen [$^1O_2$] which is the major cytotoxic agent during PDT. The efficiency of PDT can be correlated to the reacted singlet oxygen. SOED methods were used to calculate reacted singlet oxygen in PDT. Explicit PDT dosimetry can be performed in pre-clinical and clinical applications. In most studies, it is aimed to measure light fluence and photosensitizer concentration. Current methods focus to measure tissue oxygenation concentrate but they are still in pre-clinical stage.

In this part of the study, the goal is to calculate the effect of pulse duration, pulse frequency, and light fluence on ^{1}O2 generation. In the studies given in references [23,46,52], it is validated that [$^3O_2$] consumption and [$^1O_2$] generation can be determined by adjusting the pulse mode radiation parameters. Based on type II processes, equations for the reactions have been established with photophysical parameters [44–46]. Differential equations can be used to calculate time-dependent interactions of singlet oxygen concentration. Therefore, a set of differential equations valid over timescales from a few seconds to hours can be used to describe the interactions of singlet oxygen concentration [^{1}O2], photosensitizer concentration [S_0] and ground-state oxygen concentration [$^3O_2$] for in vitro scenario with the parameters defined in reference [46]. In vivo, photophysical parameters for many photosensitizers were also reviewed in reference [46] and also singlet oxygen $^1O_2$ generation macroscopic models were introduced in various researches [47–51]. However, these calculations are valid for continuous mode (CW) applications. For pulse modes there are some researches aim to calculate singlet oxygen concentration with the aid of key components parameters.

Singlet oxygen production threshold values were introduced with a set of differential equations given in Eq.s (1–4). $^3O_2$ consumption, photosensitizer photobleaching, and the oxygen supply rate were embedded to the equations by utilizing Refs. [23,52].

$$\frac{d[S_0]}{dt} + (\xi\sigma\frac{I([S_0] + \delta[^3O_2]}{[^3O_2] + \beta})[S_0] = 0 \quad (1)$$

Table 1
Photophysical parameters for pulsed mode methylene blue solution for experiments.

Photophysical parameters	
[S0]	The ground state photosensitizer concentration
I	Tight fluence rate,
$[^3O_2]$	Triplet oxygen concentration
$[^1O_2]$	Singlet oxygen concentrations
ξ	The oxygen consumption rate per light fluence rate (mW cm-2) per photosensitizer concentration (μM)
β	The ratio of the triplet photosensitizer decay rate to the rate of the triplet photosensitizer quenching by $^3O_2$
δ	Low photosensitizer concentration correction term
σ	The photosensitizer photo bleaching parameter
g	Maximum oxygen supply rate and, SA oxygen consumption rate

$$\frac{d[^3O_2]}{dt} + \left(\xi \frac{I[S_0]}{[^3O_2] + \beta}\right)[^3O_2] - g\left(1 - \frac{I[^3O_2]}{[^3O_2](t=0)}\right) = 0 \quad (2)$$

$$\frac{d[^1O_2]}{dt} - (\xi \frac{I[S_0]}{[^3O_2] + \beta})[^3O_2] = 0 \quad (3)$$

$$[^1O_2]_{cumulative} = \int (\xi \frac{I[S_0]}{[^3O_2] + \beta})[^3O_2]dt \quad (4)$$

The parameters required in the above equations are given in Table 1.

The ξ, β, δ, σ, g parameters and methylene blue values are obtained from references [48,52]. These parameters were used to acquire instantaneous singlet oxygen photon count level for each pulse and to calculate cumulative singlet oxygen concentration for whole experiment. All calculations were performed in Matlab environment. (Matlab MathWorks, Natick, MA, USA).

2.3. Experimental procedure

In this subsection, five different experiments were carried out for quantitative measurement of photon count that effects the disinfection outcome. These experiment can be summarized as given in Fig. 1.

In the first and second experiments, methylene blue solutions were investigated without any bacteria to specify the PS limitations and photon count viability. In these experiments, 1%, 0.5 %, 0.25 %, 0.125 % methylene blue with distilled water solution was used. The pulse and super pulse modes were applied for the first and second experiments, respectively. Then, the irradiation effects of the modes on methylene blue solutions were compared with each other. During the experiments, it was observed that the lower concentrations (0.25 % and 0.125 %) cannot be distinguished from each other for pulse and super pulse modes. The measured singlet oxygen concentrations with SOLD method for these concentrations are low. So, these cases were excluded for other experiments.

In the third and fourth experiments, methylene blue solutions were investigated with 100 ul bacteria. During these experiments, the concentration of methylene blue with distilled water solution were 1%, 0.5 %. The third and fourth experiments were performed to examine the effect of radiation modes on S.A for AmPDT application. After the experiments, bacteria solutions were cultured in the blood agar Petri dishes. 30 m, 1 h and 6 h examinations were performed for qualifying the survival SA.

In the fifth experiment, 1%, and 0.5 % methylene blue with distilled water solution were used. In this experiment, a simple oxygen supply mechanism, an air pump motor, was used in the environment to generate a constant airflow through the methylene blue solution. By this set up, the airflow effect on the singlet oxygen concentration was evaluated for methylene blue solutions.

In the all experiments, the wavelength of the LED light source was 660 ± 10 nm. The beam profile of the light source was uniform. The

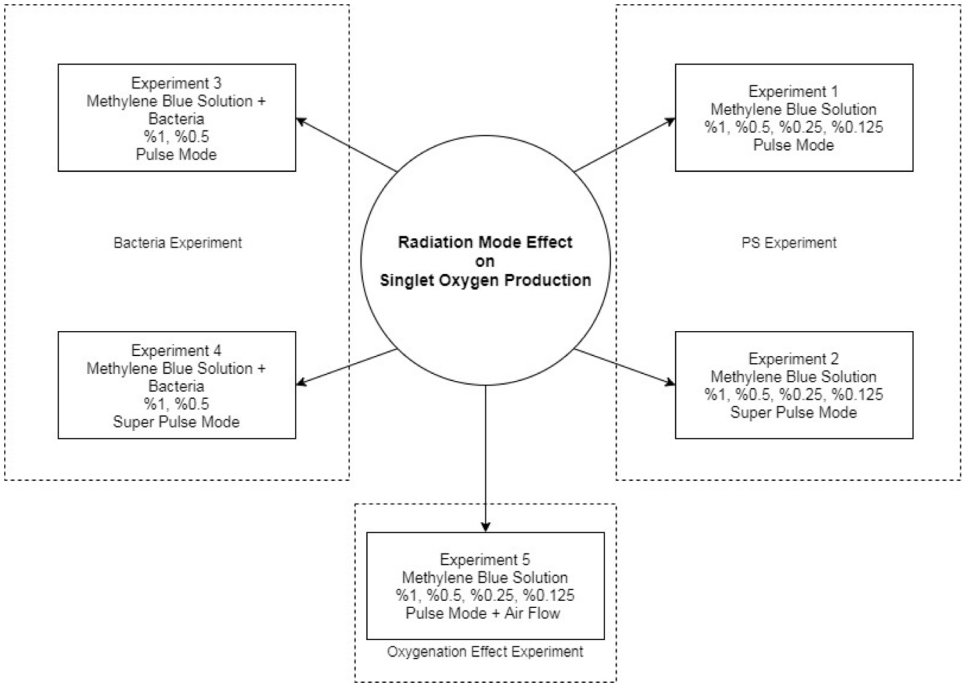

Fig. 1. The experimental setup of the study.

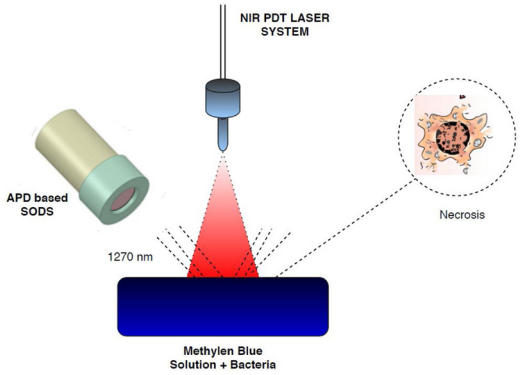

Fig. 2. Experimental setup for methylene blue solution with bacteria case.

Table 2
The radiation parameters of the performed experiments.

Radiation Parameters	
Wavelength (nm)	660 ± 10 nm
Mode	Pulse Mode, Super Pulse Mode
Power Output	100 mW, 300mW
Exposure time (s)	200 s, 200 s (off time included)
Energy density (J/cm^2)	10 (J/cm^2)
PS	Methylene Blue

difference between center and the outside of the light beam optic power was 0.1 mW. The LED light source was capable of working in the various pulse frequencies from DC to 20 MHz. For the pulse mode and super pulse mode, on-off time was kept at 50 us and 200 ns, respectively. The optic power validation was made with power meter and spectrometer (PM100 and C series spectrometer, Thorlabs, Germany). The SODS system, which is an avalanche photodiode based embedded system having signal recording capability, was used to measure the singlet oxygen illumination at 1270 nm. The experimental setup was illustrated in Fig. 2 and the radiation parameters for performed experiments were given in Table 2.

By utilizing the designed setup given in Fig. 2, the amount of photon for each pulse and for the whole experiment can be recorded. The photon counts for each pulse and the whole system can be calculated via SOED and can be measured by SOLD. Then, the achieved results can be compared to evaluate the performance of these dosimetry systems. If the photon counts per pulse decreases, the treatment may become less effective due to hypoxic environment even though the PS and light were present. Thus, the amount of singlet oxygen for each pulse and complete treatment were attained with SOLD and SOED methods.

3. Results

In this section, the results of the performed experiments were given. During the experiments, methylene blue solution was investigated with the aid of optical scattering and absorption coefficients. The photosensitizer solution was examined for the time dependency of oxygen [^{3}O2], and PS concentration [S0], under pulse and super pulse radiation modes at 660 nm. By this way, limits of the solution were determined. Also, singlet oxygen time-dependent cumulative concentration was calculated with SOED.

Methylene blue photo physical parameter values for in vitro macroscopic modeling were used in according with the existing studies [19,46]. The calculated values with SOED method have been demonstrated in Fig. 3.

The light fluence rate used in the calculations were φ_0 = 100 mW/cm^2 for methylene blue concentration with the aid of the PS extinction coefficient. The limit values for methylene blue solution without bacteria and oxygenation were demonstrated in Fig. 3 for different percentages of PS. With SOED method, calculated values for cumulative singlet oxygen concentration was between 7 − 8 mM. Five different calculation was made by changing the values within the 0.01 % error margin for mimicking the changes in real environment and the values averaged for achieving more accurate calculations. The necessary parameters for methylene blue mediated PDT for in vitro studies were validated with SOED calculations (Fig. 3). The consumption rate of [S0] per PDT dose was used to determine a more accurate PDT treatment modality.

3.1. SOED/SOLD photon count comparison

The amount of singlet oxygen produced in the methylene blue solutions was calculated using SOED and the obtained results were compared with determined singlet oxygen luminescence level by SOLD method. The correlation between the instantaneously produced singlet oxygen amount (singlet oxygen count after each pulse) and cumulatively produced singlet oxygen amount (overall singlet oxygen count) was investigated. The integration of all instantaneous singlet oxygen count was utilized for achieving the cumulative oxygen amount during the entire illumination period. The correlation between SOED and SOLD methods is demonstrated in Fig. 4. In Fig. 4 (a), SOLD results per accumulation time (200 us) was shown and Fig. 4 (b) illustrates the cumulative SOLD counts over the entire treatment (400 s).

In PDT, overall singlet oxygen production rate is highly dependent on the oxygen consumption rate. The oxygen consumption rate can be reduced either by reducing the photosensitizer concentration (but it is not optimal due to the drug photobleaching) or by reducing the average fluence rate. There are two ways to decrease the average fluence rate by applying a CW mode with a reduced fluence rate. The first way is to apply a CW mode and the second way is to create a pulsed irradiation mode [52]. Therefore, during the experiments the efficiency of the pulse modes is analyzed. *The first and second experiments* were performed to investigate the irradiation effects of pulse and super pulse modes on 1% and 0.5 %. methylene blue solutions. In this experiment limit values of the PS are considered. *The third and fourth experiments* were carried out to inspect the pulse and super pulse modes' effects on 1% and 0.5 % methylene blue solutions including bacteria. After the experiments, bacteria solution was cultured in a blood agar Petri dishes. 30 m, 1 h and 6 h analysis was made to determine the amount of surviving SA. *In the fifth experiment,* a simple experimental setup including a proper airflow is constructed to evaluate the re-oxygenation of methylene blue solution. For this purpose, 1% and 0.5 % methylene blue bacteria solutions, which provides distinguishable outputs, are used. The measurements in the experiments were made with SODS system designed by the authors [53].

Figs. 4 and 5 are plotted to show the variation of singlet oxygen concentration over time. The experiments were repeated five times with the same procedure and data were averaged to plot Fig. 4 and Fig. 5. The nanosecond-level first maximum point, which is resulted from the light source noise, was neglected while calculating the cumulative singlet oxygen concentration (Fig. 4a and Fig. 5a). Due to higher instantaneous optic power, super pulse provides higher instantaneous singlet oxygen photon count as expected.

The singlet oxygen ($^1O_2$) generation, in other words the $^3O_2$ consumption values for the first and second experiments are illustrated in Fig. 4. Fig. 5 gives the singlet oxygen ($^1O_2$) generation, in other words the $^3O_2$ consumption values for the third and fourth experiments. In these figures, the goal is to show the effect of pulse and super pulse modes on singlet oxygen generation which gives a valuable information about the efficiency of PDT. As can be seen from Fig. 4, the radiation modes have a significant effect on singlet oxygen concentration for 1% and 0.5 % methylene blue solutions. Fig. 5 proves that the radiation modes also affect the singlet oxygen concentration for 1% and 0.5 % methylene blue solution including bacteria. The irradiation in the pulse mode is defined by a peak fluence rate, the pulse duration and the pulse

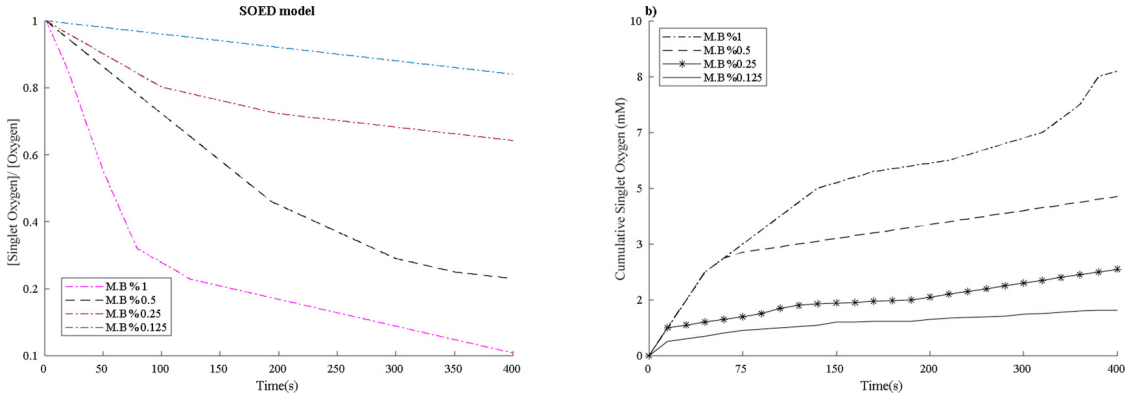

Fig. 3. SOED method calculations, a) The proportion of calculated $[^3O_2]$ values and ground state oxygen $[^3O_2]_0$ amount over time and b) volume-averaged $[^1O_2]_{rx}$ amount over time.

repetition period. The waiting period between pulses enables the oxygen concentration increase in the cells due to the oxygen supply. In pulse mode, the system has shown that cumulative singlet oxygen $^1O_2$ concentration was increased in accordance with the existing studies [52]. Super pulse mode irradiation is characterized by a peak fluence rate and the pulse duration. The optic power was delivered to the target with relatively small pulses. The penetration depths of the light in water-based solutions, bacteria solution environment and tissue are relatively higher with the super pulse mode. From the figures, we can say that the instantaneous and cumulative singlet oxygen concentrations increased by preferring super pulse radiation mode during the experiments. The increased amount of instantaneous singlet oxygen production gives rise to necrosis of more cells. The cumulative singlet oxygen concentration augmentation is an important indicator of a successful treatment.

Consequently, the singlet oxygen production level of the super pulse mode was relatively higher than that of the pulse mode under equal light energy case. For the third and fourth experiments, the bacteria solution was cultured for post-treatment and pre-treatment. Inactivated SA bacteria percentage was shown in Table 3. According to the Table 3, super pulse mode achieves a more successful AmPDT then pulse mode.

As discussed before, in the fifth experiment a simple airflow was located around the methylene blue solution. The measurements show that the airflow is not influential in producing singlet oxygen during the experiments. In the first four experiments, instantaneous remarkable singlet oxygen production decreased after ~5000 pulses due to PS extinction and hypoxia. However, in the fifth experiment, the instantaneous singlet oxygen production decreased after ~5550 pulses. As a result of this, for the fifth experiment, cumulative singlet oxygen concentration was slightly higher than that of the other experiments.

In addition to the singlet oxygen calculations, the amount of cumulative singlet oxygen (mM) measured by SOED and SOLD methods were compared for all experiments during 400 s. The main reason for choosing the period of treatment as 400 s is to apply 10 J energy level during this time which is reasonable disinfection time for SA AmPDT applications [19,46,52,54]. The obtained results were demonstrated in Fig. 6. The SOLD method measurements were made for pulse and super pulse modes. The average difference between the measurements obtained by SOED and SOLD (for pulse and super pulse modes) methods was about 7–10 % ± 0.5. This difference was in the acceptable error margin for understanding the treatment outcome. Thus, SOED model can be almost as a good choice as SOLD for laboratory experiments. However, for exact and real-time information SOLD based singlet oxygen measurement system was the prior choice. The success of SOED model can be proven after certain amount of time.

In conclusion, the singlet oxygen $^1O_2$ generation is more effective in super pulse mode than pulse mode at relatively higher doses. Locating a proper airflow around the experiment environment can increase the cumulative singlet oxygen concentration. Therefore, the effective radiation modes and simple oxygenation techniques can be used for AmPDT applications. However, SOLD based instruments are quite expensive and SOED based methods cannot give instantaneous information about the treatment. Therefore, the dosimetry model needs to be further improved by the researchers.

4. Discussion

At high fluence rates, the photodynamic process can be super effective about producing $^1O_2$ in the presence of high $^3O_2$. This is not possible in real biological systems due to the finite oxygen diffusion and

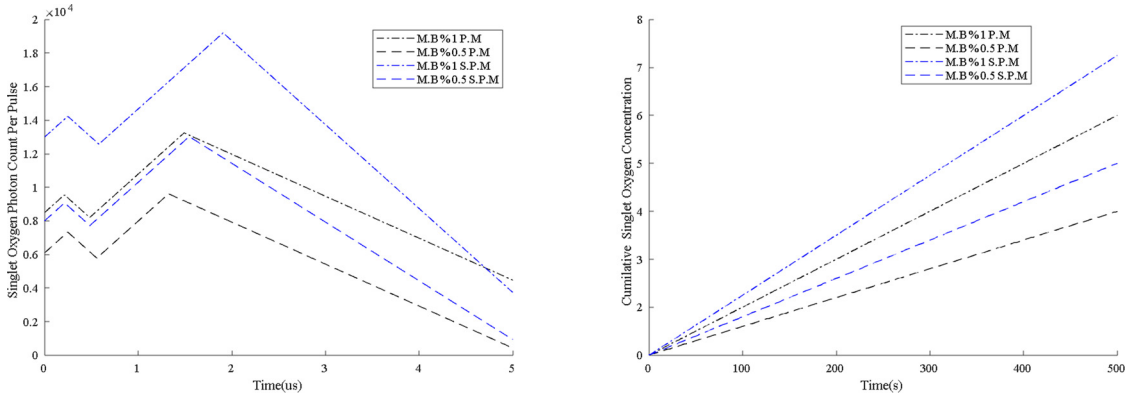

Fig. 4. Instantaneous singlet oxygen concentration after per pulse (a), and cumulative singlet oxygen concentration (mM) after irradiation time for the first and second experiments(b).

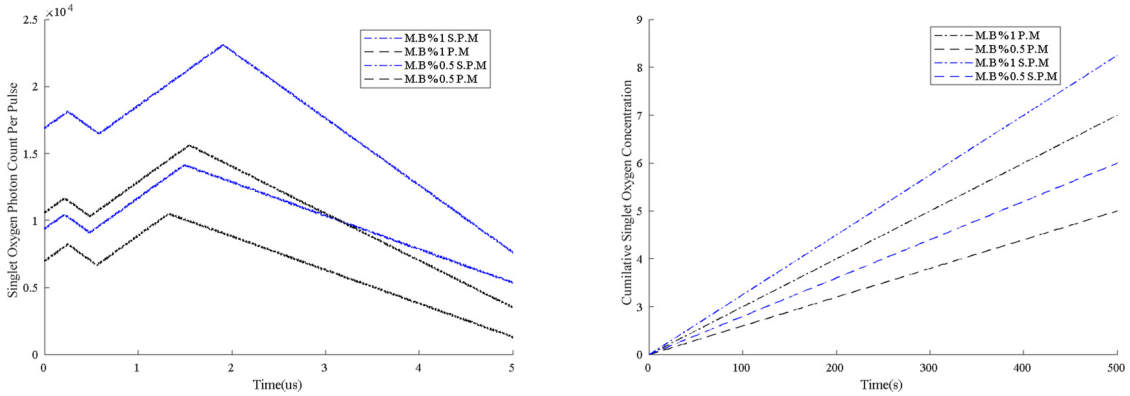

Fig. 5. Instantaneous singlet oxygen concentration after per pulse (a), and cumulative singlet oxygen concentration (mM) after irradiation time for the third and fourth experiments (b).

Table 3
SA post-treatment percentages.

Medium/Mode	Inactivated Bacteria (%)	Survival Fraction (%)
After 10 min.		
M.B %0.5 Pulse	65.32 ± 3.98	34.68 ± 3.98
M.B %1 Pulse	69.56 ± 4.78	30.44 ± 4.78
M.B %0.5 Super Pulse	66.45 ± 3.58	33.55 ± 3.58
M.B %1 Super Pulse	72.87 ± 4.89	27.13 ± 4.89
After 30 min.		
M.B %0.5 Pulse	72.23 ± 3.98	27.77 ± 3.98
M.B %1 Pulse	76.12 ± 4.78	23.88 ± 4.78
M.B %0.5 Super Pulse	72.33 ± 3.58	27.67 ± 3.58
M.B %1 Super Pulse	79.65 ± 4.89	19.35 ± 4.89
After 1 h.		
M.B %0.5 Pulse	84.23 ± 2.55	15.77 ± 2.55
M.B %1 Pulse	87.12 ± 3.97	12.88 ± 3.97
M.B %0.5 Super Pulse	86.65 ± 4.78	13.45 ± 4.58
M.B %1 Super Pulse	89.33 ± 4.58	10.77 ± 4.78
After 6 h.		
M.B %0.5 Pulse	88.22 ± 2.55	11.88 ± 1.98
M.B %1 Pulse	95.23 ± 1.98	05.77 ± 2.55
M.B %0.5 Super Pulse	90.65 ± 2.89	09.45 ± 1.12
M.B %1 Super Pulse	98.35 ± 1.12	01.65 ± 2.89

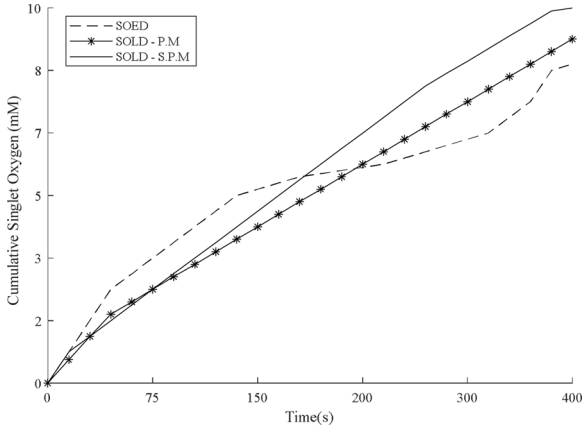

Fig. 6. The comparison of average cumulative singlet oxygen amount calculated by SOED method and measured by SOLD method for pulse and super pulse modes.

solubility. Application of low fluence rates results in the higher cumulative concentrations of $^1O_2$ and prolonged treatment time due to reduced consumption of $^3O_2$. However, even in the laboratory, the experiments cannot provide good results because of the limited oxygen concentration on the target environment [54,55]. With appropriate radiation modes, treatments that does not cause hypoxia and thermal damage on the target tissue must be investigated. There are several new generation PDT light sources developed in different modes to prevent thermal damage to the surrounding tissue hypoxia in the target environment[67,68]. The light sources with pulse and super pulse modes and macroscopic model dosimetry methods have been widely used in various research areas [52,56–58].

Zoreca and friends studied about the laser super pulses to increase penetration depth for dermatological applications [59]. In another study, the penetration depth of laser at 1030 nm is investigated in nonlinear optical microscopy of human and animal skin [60]. The difference between the penetration depth of 810 nm CW and 904 nm super pulse radiation mode is considered in the study given with [61]. According to the study, super pulse mode provides relatively deeper penetration depth even though the percentage is low and it can be used for PDT applications. In [62], it is verified that the flat wart and facial black dots can be healed faster with super pulses. In [52], Vladimir and friends examined the effect of the pulse modes on re-oxygenation of the tissues during PDT, and in [63] Vinnichenko verified the deeper penetration effect of super pulses on ablation, cut and coagulation in the water-based solution and tissue. In the light of the literature: i) PM and SPM modes can be used for clinical PDT applications to prevent the thermal damage raised during the treatment and to improve the re-oxygenation of the target environment, ii) SPM could be used to deliver relatively high energy in a short amount of time and to provide deeper penetration to reach deeply infected tissues or bacteria solutions, and iii) Higher singlet oxygen production was an important aspect of the treatment outcome and can be achieved by developing new illumination modes.

In this study, the effect of radiation modes on singlet oxygen production was investigated with different dosimetry methods. In the Experiments 1, 2, 3, and 4, instantaneous and cumulative singlet oxygen concentrations are measured for different experimental setups. The amount of singlet oxygen generation and oxygen consumption were illustrated for pulse and super pulse radiation modes. According to the obtained results, radiation modes had a significant effect on the singlet oxygen generation and pulse and super pulse modes can be used for more successful treatment. Super pulse mode provided 3–10 % more singlet oxygen concentration than pulse mode and had a relatively higher bacteria death in vitro (Table 2). With simple re-oxygenation technique as implemented in Experiment 5, it was possible to increase the cumulative singlet oxygen count and maintain the instantaneous singlet oxygen photon count for a longer period of time. It is proven that with the aid of radiation mode and oxygen supply mechanism, the performance of the AmPDT can be augmented. These techniques could be used for the treatment of cancer, disinfection, infected wound for diabetic patients, and infected prosthetics [78,79]. However, the closed loop PDT treatment dosimetry methods needs to be further improved.

Production of singlet oxygen was determined by considering several

factors. At first, the initial photosensitizer concentration and tissue optical properties must be determined by interstitial fluorescence measurement and a light dosimetry system. There are various systems for SOED measurements in the literature. Among them, the computational speed of the macroscopic model is generally fast and can be used for clinical applications for any heterogeneous light source and photosensitizer distribution. The explicit dosimetry of in vivo photosensitizer concentration, target environment optical properties and oxygenation must be taken into account for more accurate dosimetry quantity. In the proposed study, by considering the oxygen consumption rate, the SOED model was improved for AmPDT. The model was reduced the SOLD-based measurement uncertainties, and minimized the variation of singlet oxygen concentration by providing the information about singlet oxygen capabilities (Fig. 3) of the solution.

In recent years, several SOLD systems were developed. Gemmel and his research group monitored singlet oxygen luminescence with superconducting nanowire single-photon (SNSPD) and then they improved the proposed SNSPD system by using a single-photon avalanche diode detector (SPAD) [64,65]. In Ref. [66], Boso and his friends designed a practical setup based on a negative-feedback avalanche diode (NFAD) detector. The PMTs were widely used to detect singlet oxygen luminescence [69–72]. The newly developed SPADs started to be used in the singlet oxygen detection studies due to their higher detection efficiency, lower noise sensitivity, compact form factor, stability, ease-of-use, and the possibility to be gated [73–77]. However, all system mentioned above requires a complex electronic circuitry, stable distance placement, and complex mathematical calculations. The usage of the systems in clinic is still hard to accomplish. Furthermore, different technologies utilized in the singlet oxygen measurement systems does not give same photon count for the same cases.

The main idea behind the presented study was sparkled from the study given in [20]. In this study the authors were compared direct SOLD measurements with SOED calculations for phantoms using photofrin and SPAD. The amount of oxygen and PS concentration were compared with SOED predictions to validate the reliability of SOED model. Then, they showed that there was a direct correlation between the measurements obtained by SOLD and SOED methods even though having a singlet oxygen cumulative discrepancy. In the proposed study, we also verified the existence of direct correlation between SOED and SOLD methods **for pulse and super pulse radiation modes** (Fig. 6). Furthermore, the measurements in the experiments were made with SODS system designed by our research group [53]. Radiation mode, methylene blue concentration and SA bacteria were the variables of our experiments.

However, we observed that the cumulative photon count was not important for monitoring the success of the treatment. Because the amount of the cumulative singlet oxygen can change according to the used system and slightest movements can change the amount of singlet oxygen detected by the sensors. These systems are hypersensitive systems and useful in controlled laboratory environment. Also, the sensitivity, signal to noise ratio, measurement distance and the diameter of the sensing system, etc. are crucial for calculation of the photon number. The 1270 nm light scattering inside the target environment is an important aspect that can easily change with the geometry of the targeted infected or cancerous tissue. Therefore, maintaining the singlet oxygen luminescence signal shape for a longer period of time could be more crucial than getting the exact number of photon count.

Finally, in the light of our observations we suggest that the idea of initial signal shape-based singlet oxygen luminescence dosimetry might be a good candidate for clinical usage. The main purpose of the method is to maintain the averaged first set of measured signal shape for a longer period of time with the aid of SOED calculated cumulative and instantaneous values. By utilizing signal processing techniques and initial signal shape-based singlet oxygen luminescence dosimetry method can be used for developing embedded singlet oxygen detection system. The developed system can be combined with the light sources to design a closed-loop dosimetry method for PDT. By using these methods compact handheld singlet oxygen measurement systems can be developed and used more easily in the clinical area. Our system and our method can be easily used in prosthetic disinfection during surgery without disrupting the flow of the operation.

5. Conclusion

PDT is a prominent treatment used for infections, cancers and localized diseases [1–4]. The application of PDT in the clinical setting highly dependent on dosimetry metrics. Light fluence rate, tissue oxygenation, and photosensitizer distribution interactions were investigated to predict the treatment efficiency. PDT dose is currently being used as a PDT dosimetry quantity, which is calculated with light fluence and photosensitizer concentration. However, the oxygen concentration was not absolutely taken into account before. In this study, an improved dosimetry model, that considered the effect of oxygenation and firstly used in AmPDT application, is proposed. Production of singlet oxygen was determined by considering several factors. At first, the initial photosensitizer concentration and tissue optical properties must be determined by interstitial fluorescence measurement and a light dosimetry system. In the proposed study, by considering the oxygen consumption rate, the SOED model was improved for AmPDT. The model was reduced the SOLD-based measurement uncertainties, and minimized the variation of singlet oxygen concentration by providing the information about singlet oxygen capabilities of the solution. We also verified the existence of direct correlation between SOED and SOLD methods for pulse and super pulse radiation modes. Furthermore, the measurements in the experiments were made with SODS system designed by our research group [53]. Radiation mode, methylene blue concentration and SA bacteria were the variables of our experiments.

One of the targets of the study was to show the effect of pulse and super pulse modes on singlet oxygen generation which gives a valuable information about the efficiency of PDT. The obtained results showed that the instantaneous and cumulative singlet oxygen concentrations increased by preferring super pulse radiation mode during the experiments. The increased amount of instantaneous singlet oxygen production gives rise to necrosis of more cells. The cumulative singlet oxygen concentration augmentation is an important indicator of a successful treatment. Finally, we observed that the cumulative photon count was not important for monitoring the success of the treatment. Because the amount of the cumulative singlet oxygen can change according to the used system and slightest movements can change the amount of singlet oxygen detected by the sensors. Therefore, maintaining the singlet oxygen luminescence signal shape for a longer period of time could be more crucial than getting the exact number of photon count. In the light of our observations, for our future studies, we suggest that the idea of initial signal shape-based singlet oxygen luminescence dosimetry might be a good candidate for clinical usage.

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