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Explicit macroscopic singlet oxygen modeling for benzoporphyrin derivative monoacid ring A (BPD)-mediated photodynamic therapy

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Abstract

Photodynamic therapy (PDT) is an effective non-ionizing treatment modality that is currently being used for various malignant and non-malignant diseases. In type II PDT with photosensitizers such as benzoporphyrin monoacid ring A (BPD), cell death is based on the creation of singlet oxygen ($^1\text{O}_2$). With a previously proposed empirical five-parameter macroscopic model, the threshold dose of singlet oxygen ($[^1\text{O}_2]_{\text{rx,sh}}$) to cause tissue necrosis in tumors treated with PDT was determined along with a range of the magnitude of the relevant photochemical parameters: the photochemical oxygen consumption rate per light fluence rate and photosensitizer concentration (ξ), the probability ratio of $^1\text{O}_2$ to react with ground state photosensitizer compared to a cellular target (σ), the ratio of the monomolecular decay rate of the triplet state photosensitizer (β), the low photosensitizer concentration correction factor (δ), and the macroscopic maximum oxygen supply rate (g). Mice bearing radiation-induced fibrosarcoma (RIF) tumors were treated interstitially with a linear light source at 690 nm with total energy released per unit length of 22.5–135 J/cm and source power per unit length of 12–150 mW/cm to induce different radii of necrosis. A fitting algorithm was developed to determine the photochemical parameters by minimizing the error function involving the range between the calculated reacted singlet oxygen ($[^1\text{O}_2]_{\text{rx}}$) at necrosis radius and the $[^1\text{O}_2]_{\text{rx,sh}}$. $[^1\text{O}_2]_{\text{rx}}$ was calculated based on explicit dosimetry of the light fluence distribution, the tissue optical properties, and the BPD concentration. The initial ground state oxygen concentration ($[^3\text{O}_2]_0$) was set to be 40 μM in this study. The photochemical parameters were found to be $\xi = (55 \pm 40) \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $\sigma = (1.8 \pm 3) \times 10^{-5} \mu\text{M}^{-1}$, and $g = 1.7 \pm 0.7 \mu\text{M s}^{-1}$. We have taken the literature values for $\delta = 33 \mu\text{M}$, and $\beta = 11.9 \mu\text{M}$. $[^1\text{O}_2]_{\text{rx}}$ has shown promise to be a more effective dosimetry quantity for predicting necrosis than either light dose or PDT dose, where the latter is simplistically a temporal integral of the products of the photosensitizer concentration and light fluence rate.

Keywords

Photodynamic therapy; Explicit dosimetry; Singlet oxygen; Benzoporphyrin derivative monoacid ring A (BPD); *In vivo* mouse study

1 Introduction

Photodynamic therapy (PDT) has been extensively studied as an effective treatment modality for various easily accessible lesions, such as head and neck cancers, esophageal cancers, microinvasive lung cancer, and skin lesions such as premalignant actinic keratosis [1–4]. Benzoporphyrin derivative monoacid ring A (BPD, trademark Visudyne®, Valeant Pharmaceuticals North America LLC, Bridgewater, NJ), is a commonly used photosensitizer that has been approved by the U.S. Food and Drug Association (FDA) in 2000 for the treatment of wet age-related macular degeneration [5]. In a typical type II photodynamic process, the excited singlet state BPD will undergo intersystem crossing to the triplet state, which will then transfer its energy to ground state molecular oxygen ($^3\text{O}_2$) that is present and generate highly reactive singlet oxygen ($^1\text{O}_2$). PDT is a targeted treatment that destroys malignant cells with fewer side effects than conventional treatments such as surgery, chemotherapy, and radiation [1–3]. However, widespread clinical use of PDT is still a challenge due to the complex dynamic interactions between light, photosensitizer, and $^3\text{O}_2$. A well-defined single dosimetric quantity for PDT that is able to predict clinical outcomes would be beneficial.

PDT dose is a quantity that is simplistically a temporal integral of the product of the local photosensitizer concentration and light fluence rate (ϕ). This quantity is a good indicator of treatment outcome in well-oxygenated conditions [6]. During PDT treatment, hypoxia can occur for high ϕ and results in less effective PDT [7]. One can measure the $^1\text{O}_2$ production directly for type II photodynamic processes using the singlet oxygen luminescent (SOL) signal at 1270 nm [8–10]. However, singlet oxygen luminescence dosimetry (SOLD) is challenging because of the weak SOL signal and short lifetime (300–800 ns) due to rapid interactions between $^1\text{O}_2$ and biological acceptors, thus it is difficult to perform SOLD in clinical settings [8–10]. An explicit dosimetry model was suggested to calculate the reacted singlet oxygen concentration ($[^1\text{O}_2]_{\text{rx}}$) [6,11]. This macroscopic model involves five photochemical parameters with a set of PDT kinetic equations, which can be applied to any clinical treatment geometry.

This study is the first such attempt, to our knowledge, to optimize the values of the relevant photochemical parameters (ξ , σ , δ , β , and g) for *in vivo* BPD-mediated PDT with a 3-hour drug-light interval (DLI) using the macroscopic model. A series of PDT treatments with different total energy released per unit length (22.5–135 J/cm) and light source strengths (12–150 mW/cm) was used to induce different radii of necrosis in radiation-induced fibrosarcoma (RIF) tumors on mice. Based on data from control mice (with no BPD or light exposure), the PDT-induced necrosis was determined by subtracting the control necrosis radius from the final necrosis radius. Spatially and temporally resolved $[^1\text{O}_2]_{\text{rx}}$ was calculated based on explicit dosimetry of the light fluence distribution, the tissue optical

properties, and the BPD concentration. PDT dose and $[^1\text{O}_2]_{\text{rx}}$ was correlated to the radius of necrosis due to PDT and compared as dosimetric quantities.

2 Theory and Methods

2.1 Tumor Model

RIF cells were cultured and 30 μl were injected at 1×10^7 cells/ml in the right shoulders of 6–8 week old female C3H mice (NCI-Frederick, Frederick, MD), as described previously [6,12,13]. Animals were under the care of the University of Pennsylvania Laboratory Animal Resources. All studies were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Tumors were treated when they were ~8–10 mm in diameter. The fur of the tumor region was clipped prior to cell inoculation, and the treatment area was depilated with Nair (Church & Dwight Co., Inc., Ewing, NJ) at least 24 h before measurements. Mice were provided a chlorophyll-free (alfalfa-free) rodent diet (Harlan Laboratories Inc., Indianapolis, IN) starting at least 10 days prior to treatment to eliminate the fluorescence signal from chlorophyll-breakdown products, which have a similar emission range to the BPD fluorescence spectra which is used to determine the concentration of BPD in the tumor interstitially using catheters (Fig. 1).

2.2 Measurement of the Tissue Optical Properties and Interstitial BPD Concentration

Interstitial fluorescence measurements were made by inserting a side-cut fiber into one of the two catheters that were inserted into the tumor. The side-cut fiber was connected to a 405 nm laser (Power Technology Inc., Little Rock, AR), a dichroic beam splitter, and a multichannel CCD spectrograph (InSpectrum, Princeton Instruments, Trenton, NJ). Collected spectra were analyzed using single value decomposition (SVD) fitting [14]. Spectra were measured both before and after treatment to investigate the effects of and relationship between photobleaching and outcome. The *in vivo* photosensitizer concentration was obtained by comparing the *in vivo* BPD fluorescence with that of phantoms with known BPD concentrations. An empirical correction factor was obtained from phantom experiments with known constant BPD concentrations and varying absorption and scattering coefficients (μ_a and μ_s'). A set of experiments in tissue-simulating phantoms containing Intralipid (Fresenius Kabi, Uppsala, Sweden) as a scatterer and Parker Quink (Parker Pen Company, New Haven, East Sussex, England) as an absorber were designed. μ_a and μ_s' were varied for a fixed BPD concentration (0.25 mg/kg), and fluorescence spectra were analyzed with SVD [14] to determine the spectral component magnitudes for BPD and the autofluorescence from the 690 nm excitation laser light. The data was then used to determine the empirical optical property correction factor for the fluorescence method used to determine the PS concentration (Fig. 2 (a)). A more accurate method would involve knowledge of the optical properties at the excitation wavelength (405 nm) as well as the emission wavelength (690 nm) [15], the former is beyond the current fluorescence spectroscopy range. A multiplicative correction factor of the following form can be determined [15]:

$$CF = a \frac{\exp(b \cdot \mu_{eff})}{\mu_s'} \quad (1)$$

The raw fluorescence SVD is corrected by multiplying it with CF to get corrected $SVD(SVD_{corr})$. The values of a and b were optimized so that SVD_{corr} for phantoms with the same concentration of BPD were matched (Fig. 2 (a)). Upon optimization, it was found that $a = 1.2$ and $b = 0.5016$. A separate tissue-simulating phantom with fixed optical properties ($\mu_a = 0.7 \text{ cm}^{-1}$ and $\mu_s' = 10.1 \text{ cm}^{-1}$) and varying concentrations of BPD (μM) were used to determine a calibration curve for SVD_{corr} (Fig. 2 (b)).

A two-catheter method (shown in Fig. 1 (a)) was used to determine *in vivo* optical properties in the RIF tumor at the treatment wavelength [16,17]. The light fluence rate at different distances from a point source is determined using an isotropic detector moving along a catheter located at a fixed distance from the point source. The fit to this data according to the diffusion equation described elsewhere [16] can be used to determine μ_a and μ_s' .

2.3 Spectrofluorimetry for Verification of *In Vivo* BPD Concentration

In order to verify the *in vivo* photosensitizer concentrations, *ex vivo* experiments were performed on mouse tumors after injection of BPD. Measurements of BPD levels in tissue were performed based on published *ex vivo* procedures [18–20]. After the correct incubation time (3 h for BPD), tumor tissue samples were excised and immediately frozen for later use. At the time of measurement, samples were thawed to room temperature, weighed, minced, and placed in a vial with the appropriate amount of tissue solubilizer, Solvable (Packard, Meriden, CT). The samples were then heated at 50 °C in the dark for 4 h. After the solution was cooled, an equal volume of water was added, and with thorough mixing, the solution was transferred to a cuvette to be measured. The fluorescence of the solubilized samples was measured using a spectrofluorometer (FluoroMax-3, Jobin Yvon, Inc., Edison, NJ) with an excitation wavelength of 435 nm. The concentration of BPD was calculated based on the increase in fluorescence signal resulting from the addition of a known amount of BPD to each sample after its initial reading. The *ex vivo* measurements were compared to *in vivo* measurements using the method described in Section 2.2 for the same tumors (Fig. 2(c)). The good agreement between the two confirmed the accuracy of the interstitial method used *in vivo* both pre- and post- PDT.

2.4 Treatment

Mice were treated with linear source strength (LS), power released per length, of 12–150 mW/cm and total energy released per length of 24–135 J/cm. A 1-cm long cylindrical diffusing fiber (CDF) light source was connected to an 8 W, 690 nm diode laser (B & W Tek Inc., Newark, DE) via a SMA connector. Two catheters were inserted parallel with a 3-mm separation into the mouse tumor (Fig. 1 (a)). One catheter included the treatment light and was central to the tumor, and the second catheter held the isotropic detector or a side-cut fiber for optical properties or fluorescence measurements.

2.5 RIF Tumor Necrosis Measurement and Analysis

The tumors were excised 24 h after PDT and stored in formalin until time for embedding and sectioning. Tumors were sectioned at 200 μm slices perpendicular to the treatment linear source orientation and placed onto slides to be stained with hematoxylin and eosin (H & E) to see the necrotic area. Slides were then scanned digitally with a ScanScope microscope (Leica Microsystems Inc., Buffalo Grove, IL), and necrotic radii were obtained. Controls were included for each group of animals studied, with the average necrotic radius among these controls (r_0) being 1.6 mm. This was calculated by determining the necrotic area (A_c) on the digitally scanned slide and using the formula $A_0 = \pi r_0^2$. The PDT-induced necrosis for each treated mouse was determined by $r_n = r_t - r_0$, where r_t is the measured raw necrosis for treated mice. For all necrosis radii, a shrinkage factor (SF) was included due to the tumor shrinkage induced by preservation in formalin. This was determined by measuring the tumor dimensions prior to formalin fixation and after at least 24 h of fixation time. The standard error for each necrosis radius was determined by propagating the uncertainty from each source of error. The standard deviation of each individual mouse tumor necrosis radius as well as the uncertainty in radius measurement due to the ellipsoidal shape of certain tumor sections was included in this calculation. For ellipsoidal tumors, the radius was measured from the light delivery source insertion point to the edge of the necrotic area at various angles to determine the uncertainty due to this shape.

2.6 Macroscopic Singlet Oxygen Model

The macroscopic singlet oxygen model used was previously described [2]. The theory is derived from a type II PDT mechanism. The photochemical reactions can be simplified to four coupled differential equations [6,11,21–23]

$$\mu_a \varphi - \nabla \cdot \left(\frac{1}{3\mu_s} \nabla \varphi \right) = S, \quad (2)$$

$$\frac{d[S_0]}{dt} = -\frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] + \beta} \varphi [S_0] ([S_0] + \delta) \xi \sigma, \quad (3)$$

$$\frac{d[{}^3\text{O}_2]}{dt} = -\frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] + \beta} \varphi [S_0] \xi + g \left(1 - \frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] (t=0)} \right), \quad (4)$$

$$\frac{d[{}^1\text{O}_2]_{rx}}{dt} = \frac{[{}^3\text{O}_2]}{[{}^3\text{O}_2] + \beta} \varphi [S_0] \xi \quad (5)$$

where ϕ is the light fluence rate, S is the source term, and μ_a and μ_s' are the absorption and scattering coefficients, respectively. Five parameters are involved in the kinetic equations (ξ , σ , β , δ , and g). ξ is the photochemical oxygen consumption rate per light fluence rate and photosensitizer concentration under the condition that there is ample $^3\text{O}_2$ supply. σ is the probability ratio of a $^1\text{O}_2$ molecule to react with ground state photosensitizer compared to the $^1\text{O}_2$ molecule reacting with a cellular target $[A]$. β represents the ratio of the monomolecular decay rate of the triplet state photosensitizer to the bimolecular rate of the triplet photosensitizer quenching by $^3\text{O}_2$. δ is the low concentration correction, and g is the maximum macroscopic oxygen perfusion rate [6]. A summary of the parameters is listed in Table 1.

For a given value of ϕ , spatially resolved light fluence rate profiles can be constructed using Eq. (1), which will then be used in the calculation of the PDT kinetics equations (Eqs. (2)–(4)). For this study, a 1-cm CDF was used as the treatment source. From the simulation results, we can see from Fig. 3 that the light fluence rate distribution within a 5 mm radial distance with respect to the center of the linear source does not show significant difference for the case of experimentally varying optical properties.

As described previously [6,12], the optimization processes starts by solving the PDT equations using initial estimates for the modeling parameters (g , ξ , σ), along with the experimental light fluence rate, the initial oxygen concentration, and the initial photosensitizer concentration. Two of the parameters, β and δ , were fixed values based on the literature [24,25]. The result of optimization gave g , ξ , σ and singlet oxygen threshold dose $[^1\text{O}_2]_{\text{rx,sh}}$. The initial oxygen concentration was assumed to be 40 μM [26]. After calculating the time series solution for $[^1\text{O}_2]_{\text{rx}}$, this value at the radius of necrosis with the given input parameters was compared to the calculated $[^1\text{O}_2]_{\text{rx,sh}}$ by minimizing a standard deviation according to the following:

$$f = \sqrt{\frac{\sum_i^N \left| 1 - \frac{[^1\text{O}_2]_{\text{rx}}(r_i)}{[^1\text{O}_2]_{\text{rx,sh}}} \right|^2}{(N(N-1))}} \quad (6)$$

Here, N is the total number of groups or individual mice, and r_i is the measured radius of necrosis for group/mouse number i . Multi-variable optimization using the functional minimization function “fminsearch” from Matlab (Mathworks, Natick, MA) was implemented.

Error margins for the fitted parameters were determined by propagating the systemic and random error from the experiment through the fitting process. To determine the variation in the resulting parameters that were fit, over 500 combinations of initial input parameters were chosen to start the fitting. The initial estimated parameters ranged from $\sigma = (0.5-10) \times 10^{-5} \mu\text{M}^{-1}$, $\xi = (10-100) \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $g = 0.5-2.0 \mu\text{M s}^{-1}$, and $[^1\text{O}_2]_{\text{rx,sh}} = 0.5-2.0 \text{ mM}$. In each iteration, only one parameter was changed within the range presented while the others were set to the standard initial estimations ($\sigma = 5 \times 10^{-5} \mu\text{M}^{-1}$, $\xi = 85 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $g = 0.7 \mu\text{M s}^{-1}$, and $[^1\text{O}_2]_{\text{rx,sh}} = 0.7 \text{ mM}$). Each round of optimization minimized

the objective function Eq. (5) and output parameters were collected and analyzed for their maximum deviations. Final values determined from the best optimization of the objective function are presented along with their minimum and maximum ranges in Table 2.

Using the macroscopic model, $[^1\text{O}_2]_{\text{rx}}$ and PDT dose were calculated and compared to PDT induced necrosis. $[^1\text{O}_2]_{\text{rx}}$ and PDT dose at 3 mm from the CDF was used as our dose metrics, where 3 mm was chosen since it happened to be the distance used in our measurement between the two catheters. $[^1\text{O}_2]_{\text{rx}}$ and PDT dose at the necrosis radius were also calculated as second dose metrics.

Necrosis radii, along with their standard uncertainties, are presented in Table 1. The error includes the sum of squares of variations in radius measured between mice treated with the same condition as well as the systematic error of necrosis. Some of the tumor sections obtained exhibited an ellipsoidal shape due to the treatment and sectioning. The variation in necrosis due to the ellipsoidal shape was taken into account as systematic error. Standard deviation of each calculated value of fluence, PDT dose, and $[^1\text{O}_2]_{\text{rx}}$ are also presented in Table 1. Typically, 3 mice are used in each treatment group.

All fitting and simulation were performed using Matlab R2014b on an iMac OSX version 10.10.5 (processor 2.9 GHz Intel Core i5, 16 GB memory). The calculation times were in seconds for the rate equations and in minutes for the spatially coupled differential equations.

3 Results

The concentration of BPD in tumors was acquired interstitially. This method was verified with an *ex vivo* method described in Section 2.3. The results of the comparison are shown in Fig. 2. Each data point represents the average value of three separate measurements made in the same tumor using both interstitial and *ex vivo* methods. The best linear fit obtained when comparing the two was $y = 0.98x$ with $R^2 = 0.98$ (black solid line). The dashed line represents a line with a slope of 1, which would be the case if both measurements were in perfect agreement. However, the results from the comparison show that the fluorescence correction for interstitial measurements is fairly accurate.

The distribution of ϕ in tumor tissue was calculated using the diffusion equation and the linear light source characteristics. Measured optical properties in tumors were used as input parameters to see the effect of varying μ_a and μ_s' on the light fluence distribution. Fig. 3 shows the drop of ϕ/LS along the tumor depth. However, with varying measured μ_a and μ_s' , the deviation of ϕ/LS inside tumors at depths up to 3 mm was within the deviation of the measured μ_a and μ_s' (indicated by the grey region). The mean measured optical properties were $\mu_a = 0.7 \pm 0.1 \text{ cm}^{-1}$ and $\mu_s' = 11 \pm 2.4 \text{ cm}^{-1}$.

Fig. 4 (a) shows PDT-induced necrosis radius *versus* the $[^1\text{O}_2]_{\text{rx}}$ at the necrosis radius. The legend indicates each treatment condition. Fig. 4 (b) shows the model predicted PDT-induced necrosis *versus* the measured PDT-necrosis. The good correlation is an indicator of the accuracy of the optimization fit. Table 2 summarizes the photochemical parameters obtained from the fitting routine as well as their initial values for the model. The best fit estimated values (along with their standard deviation) of $\sigma = (1.8 \pm 3) \times 10^{-5} \text{ }\mu\text{M}^{-1}$, $\xi = (55$

$\pm 40) \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $g = 1.7 \pm 0.7 \text{ } \mu\text{M s}^{-1}$, and $[^1\text{O}_2]_{\text{rx,sh}} = 0.67 \pm 0.13 \text{ mM}$. The values for β and δ were held constant at $33 \text{ } \mu\text{M}$ and $11.9 \text{ } \mu\text{M}$, respectively. The grey region in Fig. 4 (a) shows the range for the singlet oxygen threshold concentration, $[^1\text{O}_2]_{\text{rx,sh}}$. The PDT dose was calculated at the necrosis radius and compared with the PDT-induced necrotic radius in Fig. 4 (c). The grey region indicates the upper and lower bounds of the fit with a 95% confidence interval. The best fit to the data was $y = 0.1708x$ with $R^2 = 0.127$, indicating that PDT dose at the necrosis radius is not well-correlated with the necrotic outcome.

Fig. 5 (a) shows the PDT dose at 3 mm vs. PDT-induced necrosis radius. The solid line represents the best fit to the data using a functional form of $y = 1802x$ with a goodness of fit of $R^2 = 0.147$. Fig. 5 (b) shows the photobleaching ratio vs. PDT-induced necrosis radius. Photobleaching ratio was calculated as $1 - ([SVD]_{\text{post}}/[SVD]_{\text{pre}})$, where $[SVD]_{\text{pre}}$ and $[SVD]_{\text{post}}$ are the measured BPD fluorescent components pre- and post-PDT. While there is a positive correlation with PDT-induced necrosis as indicated by the linear fit of $y = 2.28x$, it is not a good fit of the data since goodness of fit $R^2 = 0.436$. Fig. 5 (c) shows the $[^1\text{O}_2]_{\text{rx}}$ a depth of 3 mm vs. PDT induced necrosis. The solid line is the best fit to the data $y = 8.43/(1 + \exp(-(x - 0.92)/0.16))$ with a goodness of fit of $R^2 = 0.96$. The grey regions indicate the upper and lower bounds of the fit with a 95% confidence interval.

4 Discussion

PDT promises to be an effective treatment modality for diseases. However, clinical application of PDT has been hindered due to the complicated dosimetry [6,27]. BPD-mediated PDT has been shown to correlate well with calculated $^1\text{O}_2$ as shown in this study. A DLI of 3 h was used for this study. By this time, the drug has systemically extravasated into the tumor interstitial and cellular components [28]. With a shorter DLI, vascular-targeted PDT can be achieved, which results in a different outcome that is not presented here [28].

Currently, the common approach in clinical PDT dosimetry is based on the photosensitizer concentration that is administered to the patient and the amount of light delivered to the treatment site. This method does not account for many of the complexities that arise with PDT. If the treatment site is hypoxic, or becomes hypoxic through the course of the treatment, the expected $^1\text{O}_2$ produced will be higher than what is produced and treatment will be less effective [27,29]. As seen in the data from Table 1, photosensitizer uptake is very heterogeneous even though the administered dose is the same. This variation in photosensitizer concentration in the treatment tissue from sites to sites within the same individual (intra-tumor variation) and from individual to individual (inter-tumor variation) results in varied PDT treatment response [6,27,30]. Optical properties of the treatment tissue affect the penetration of light into the target area and are tissue-type dependent [31]. Furthermore, optical properties can be affected by the tissue and blood oxygenation, which is a key component in PDT [31–33]. All of these factors are dynamically changing during PDT, making accurate clinical dosimetry a challenge.

Singlet oxygen explicit dosimetry (SOED) is of particular interest as it involves measurement of the key components involved in PDT and modeling $^1\text{O}_2$, the major

cytotoxic agent in type-II PDT. Pre-clinical studies were performed using a murine model to determine the range of relevant photochemical parameters needed for BPD-mediated PDT explicit dosimetry. A range of source strengths and exposure times were performed on mice with RIF tumors to generate varying amounts of $^1\text{O}_2$ and induce necrosis. $[\text{}^1\text{O}_2]_{\text{rx}}$ were calculated using the macroscopic model using the information obtained from pre-PDT measurements regarding the tumor tissue optical properties (μ_a and μ_s') and the photosensitizer concentration. The distribution of ϕ was also calculated using the diffusion approximation for a linear source. Photosensitizer concentration was measured with interstitial fluorescence spectra, and the method was validated by comparing the *in vivo* and *ex vivo* measurements on separate mice.

Measured optical properties were used to calculate ϕ distribution in the tumor tissue using the light source characteristics and the diffusion equation. The first 7 treatment groups included 3 mice per treatment condition. The deviation in ϕ at depths up to 3 mm varied 20%, indicating that variations in optical properties account for less than 20% of the experimental error with consistent, long-term measurements with the same experimental set-up (see Fig. 3 (b)). Some of the measurements in individual mice made in an earlier experiment with a different batch of mice (Table 1b) shows larger deviations of ϕ (up to 40%) (see Fig. 3 (b)), which contain additional measurement uncertainties.

Fig. 4 shows the most important results of this study. The threshold dose model only works to an extent if the threshold concentration for $[\text{}^1\text{O}_2]_{\text{rx}}$ is allowed a range as shown by the grey shaded area in Fig. 4 (a). Those that do not achieve the threshold singlet oxygen dose (indicated by the dashed black line and its uncertainty as indicated by the grey area) do not exhibit any PDT-induced necrosis (data points 9, 10, 14, 15, and 16). The other points that achieve the threshold dose delineate the upper and lower bounds of uncertainty for $[\text{}^1\text{O}_2]_{\text{rx,sh}}$. By plotting the measured PDT-induced necrosis radius against the model-predicted values (the values from each $[\text{}^1\text{O}_2]_{\text{rx}}$ profile curve that intersect the $[\text{}^1\text{O}_2]_{\text{rx,sh}}$ dashed line) in Fig. 4 (b), the goodness of the macroscopic model in predicting the necrosis radius can be evaluated. A good correlation indicates a good fit. The dashed line indicates a perfect correlation and the fit to the data yields $y = 1.085x$ with $R^2 = 0.838$. The grey region indicates the 95% confidence interval of the fit. Fig. 4 (d) shows that unlike $[\text{}^1\text{O}_2]_{\text{rx}}$, PDT dose at the necrosis depth is a very poor predictor for the necrosis radius, with $R^2 = 0.127$.

The calculated $[\text{}^1\text{O}_2]_{\text{rx}}$ was fit to the *in vivo* BPD-mediated necrosis radius so that the photochemical parameters, g , ξ , and σ could be determined along with the $[\text{}^1\text{O}_2]_{\text{rx,sh}}$. The uncertainty for the resulting photochemical parameters (g , ξ , σ) was quite large based on the fitting algorithm and incorporating experimental uncertainties. However, these values have ranges large enough to include the values obtained from a previous preliminary study [34].

The parameters obtained for BPD ($\xi = 55 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $\sigma = 1.8 \times 10^{-5} \text{ }\mu\text{M}^{-1}$, and $g = 1.7 \text{ }\mu\text{M s}^{-1}$) were compared to those obtained for HPPH ($\xi = 70 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$, $\sigma = 1 \times 10^{-5} \text{ }\mu\text{M}^{-1}$, and $g = 1.5 \text{ }\mu\text{M s}^{-1}$) [21], Photofrin ($\xi = 2.1\text{--}3.7 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$ and $\sigma = 7.6 \times 10^{-5} \text{ }\mu\text{M}^{-1}$) [13,35,36], mTHPC ($\xi = 30 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$ and $\sigma = 3.0 \times 10^{-5} \text{ }\mu\text{M}^{-1}$) [13,35,36], and ALA-PpIX ($\xi = 3.7 \times 10^{-3} \text{ cm}^2 \text{ mW}^{-1} \text{ s}^{-1}$ and $\sigma = 9.0 \times 10^{-5} \text{ }\mu\text{M}^{-1}$) [13,35,36]. The value of ξ for BPD was found to be larger than that of other

photosensitizers except HPPH, which corresponds to the proportionality of ξ with the absorption coefficient and the larger absorption coefficient of BPD. The fit value of σ was found to be smaller than those of other photosensitizers, but in the same range as that of HPPH. The $[^1\text{O}_2]_{\text{rx,sh}}$ was found to be 0.67 ± 0.13 mM, which is similar to the reported value for that of Photofrin ($[^1\text{O}_2]_{\text{rx,sh}} = 0.7 \pm 0.3$ mM) [6]. The values are presented with their standard deviations.

Fig. 5 compares three dosimetric metrics PDT dose (Fig. 5 (a)), photobleaching ratio (Fig. 5 (b)), and $[^1\text{O}_2]_{\text{rx}}$ (Fig. 5 (c)) *versus* PDT-induced necrosis. The grey area shows the upper and lower bounds of the fits with a 95% confidence level. It is clear that $[^1\text{O}_2]$ is the best dosimetric indicator compared to PDT dose and photobleaching ratio, as defined by $1 - [SVD]_{\text{post}}/[SVD]_{\text{pre}}$, the $[SVD]_{\text{pre}}$ and $[SVD]_{\text{post}}$ are the BPD fluorescence [27] components before and after PDT. The solid lines in the figure represent the best fit to the data. Their goodness of fit $R^2 = 0.96, 0.436$, and 0.147 for $[^1\text{O}_2]_{\text{rx}}$, photobleaching ratio, and PDT dose, respectively. The reason PDT dose is not a good indicator may be due to ignoring the oxygen consumption during PDT. The reason that photobleaching ratio is not a very good indicator may be associated to the fact that $[S_0]$ concentration *in vivo* ($0.2\text{--}1$ μM) is much lower than $\delta = 33$ μM for BPD. $[^1\text{O}_2]_{\text{rx}}$ was calculated using the macroscopic model and incorporating information regarding the spatial distribution of ϕ based on measured tissue optical properties and the photosensitizer concentration in tissue. For this study, the initial ground state oxygen concentration ($[^3\text{O}_2]_0$) was fixed to 40 μM . However, this could be further improved in the future by performing $[^3\text{O}_2]$ measurements directly for each mouse.

For the practical application of SOED in clinical PDT, it is not generally necessary to determine the tissue optical properties in order to calculate $[^1\text{O}_2]_{\text{rx}}$. $[^1\text{O}_2]_{\text{rx}}$ can be calculated directly using the measured ϕ and PS concentration using Eq. (6), using the measured ϕ and $[S_0]$, and $[^3\text{O}_2]$ can be determined using Eqs. (4)–(5). The only other unknowns for a specific photosensitizer are the photochemical parameters, which can be found in the literature for most common photosensitizers [37].

5 Conclusion

An explicit dosimetry model for BPD-mediated PDT was investigated on mice bearing RIF tumors. Since direct measurement of $^1\text{O}_2$ concentration is difficult *in vivo*, SOED can be useful as a measure of PDT dosimetry. The photochemical parameters needed for the macroscopic modeling of $^1\text{O}_2$ were found. The threshold dose of singlet oxygen to induce necrosis in the tumor was determined by correlating the calculated $[^1\text{O}_2]_{\text{rx}}$ and the tumor necrosis induced by PDT. Correlation of PDT-induced necrosis with photobleaching ratio, PDT dose, and $[^1\text{O}_2]_{\text{rx}}$ was compared. It showed that $[^1\text{O}_2]_{\text{rx}}$ serves as a better dosimetric quantity than photobleaching ratio or PDT dose in predicting the treatment outcome. This study is important in understanding the effect of $^1\text{O}_2$ -based dosimetry for BPD-mediated PDT as well as determining the range of photochemical parameters required for SOED. A further study will be necessary to investigate the correlation between SOED calculated $[^1\text{O}_2]_{\text{rx}}$ to a more meaningful PDT treatment efficacy, such as local tumor control rather than necrosis.

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Highlights

- Photochemical parameters for macroscopic singlet oxygen modeling for BPD were obtained.
- *In vivo* threshold singlet oxygen concentration for BPD to induce necrosis was 0.67 mM.
- Four dose metrics *versus* necrosis radius as PDT outcome are compared.
- Reacted singlet oxygen concentration is the best dose predictor of necrosis radius.

Fig. 1.

(a) Treatment set-up of interstitial PDT in a mouse RIF tumor. (b) Treatment set-up schematic of interstitial PDT. One catheter is inserted centrally inside the tumor to contain the cylindrically diffusing fiber and the second catheter is inserted 3 mm away to contain the isotropic detector for light fluence profile measurements for optical properties or the side-firing fiber for obtaining fluorescence spectra. (c) Sample cross-section of tumor stained with H&E to determine necrosis radius.

Fig. 2.

(a) Fluorescence singular value decomposition (SVD) amplitude for BPD in tissue-simulating phantom experiments with different optical properties but constant BPD concentration. An empirical correction factor (*CF*) of the form described in Eq. (1) was obtained so that the corrected SVD_{corr} amplitudes were the same. (b) A calibration curve of BPD concentration (in μ M) versus SVD_{corr}. (c) Interstitially measured *in vivo* BPD concentration versus *ex vivo* measured BPD concentration. Each data point represents the average of three *in vivo* and *ex vivo* measurements performed in the same RIF tumor. The

solid line represents the best linear fit ($y = 0.99x$) to the data with $R^2 = 0.99$. The dashed line represents $y = x$.

Fig. 3.

(a) Fluence rate (in mW/cm^2) per linear source strength (LS , in mW/cm) in RIF tumor tissue for various optical properties measured in mice. Calculations were made with a linear source model. (b) The fluence rate relative to the mean fluence rate for all measured optical properties. The maximum deviation at 3 mm away from the light source is around 20%, for the first 7 treatment groups with 3 mice each, indicating that variations in optical properties account to less than 20% of the experimental error with consistent measurements.

Fig. 4.

(a) $[^1\text{O}_2]_{\text{rx}}$ versus radius for mice using Eqs. (2)–(4) and the photochemical parameters in Table 2. Data points 1–7 were obtained by averaging a group of 3 mice with the same treatment conditions while data points 8–16 were individual mice. The bold dashed black line indicates $[^1\text{O}_2]_{\text{rx,sh}}$ determined by this study (0.67 mM), and the grey region indicates the range. (b) The PDT-induced necrotic radius that is calculated by the model versus the measured PDT-induced necrotic radius. The dashed line indicates a perfect correspondence between calculated and measured data ($y = x$). The solid line is a linear fit to the data, $y = 1.085x$ with $R^2 = 0.7579$ (c) PDT-induced necrosis radius versus PDT dose calculated at the necrosis radius. The solid line shows a linear fit to the data using the functional form $y =$

$0.1708x$ with $R^2 = 0.127$. The grey area shows the upper and lower bounds of the fit with a 95% confidence interval.

Fig. 5.

PDT-induced necrosis *versus* (a) PDT dose at 3 mm, (b) photobleaching ratio, and (c) $[^1\text{O}_2]_{\text{rx}}$ at 3 mm. PDT dose was calculated for those groups of mice where there was a PDT-effect due to treatment. Photobleaching ratio was calculated by $1 - [\text{SVD}]_{\text{post}}/[\text{SVD}]_{\text{pre}}$ for those groups of mice where pre- and post-PDT BPD fluorescence components were measured. $[^1\text{O}_2]_{\text{rx}}$ was calculated for all treated groups of mice. The solid lines show the best fits to the data using the functional forms (a) $y = 0.1802x$ with a goodness of fit $R^2 = 0.147$, (b) $y = 2.28x$ with a goodness of fit $R^2 = 0.436$, and (c) $y = 8.43/(1 + \exp(-(x - 0.92)/0.16))$.

0.16)) with a goodness of fit $R^2 = 0.96$. The grey area shows the upper and lower bounds of the fits with a 95% confidence interval.

Table 1

Tissue optical properties and photosensitizer concentrations.

Treatment conditions					Optical properties		Necrosis analysis			Calculated dosimetric metrics				
Group	LS ^a (mW/cm)	Time (s)	Energy ^b (J/cm)	BPD Conc. (μM)	μ _a (cm ⁻¹)	μ _s [′] (cm ⁻¹)	Necrosis radius, r _i (mm)	Control radius, r ₀ (mm)	PDT-induced necrosis (mm)	Fluence rate at 3 mm (mW/cm ²)	Fluence rate at r _i (mW/cm ²)	PDT dose at 3 mm (μM J/cm ²)	PDT dose at r _i (μM J/cm ²)	[O ₂] _{rx} at 3 mm (mM)
(a)	Experiment 1; group 1; each treatment group contains 3 mice. Mean value and standard deviations are presented for each group													
1	12	2000	24	0.90 ± 0.14	0.8 ± 0.1	8.0 ± 0.5	2.8 ± 0.7	1.6	1.2 ± 0.7	11.5 ± 1.7	13.2 ± 1.4	5.2 ± 1.7	5.8 ± 0.9	0.6 ± 0.3
2	12	3000	36	0.71 ± 0.03	0.7 ± 0.1	9.4 ± 1.1	3.0 ± 0.7	1.3	1.7 ± 0.7	13.5 ± 2.1	15.5 ± 1.7	4.0 ± 1.1	4.6 ± 1.1	0.7 ± 0.3
3	20	1600	32	0.49 ± 0.07	0.7 ± 0.1	8.0 ± 0.5	1.9 ± 0.7	1.0	0.9 ± 0.7	21.7 ± 2.9	24.7 ± 2.5	4.8 ± 0.6	4.0 ± 0.1	0.5 ± 0.1
4	30	1020	30.6	0.98 ± 0.02	0.9 ± 0.1	7.9 ± 1	3.4 ± 0.7	1.3	2.1 ± 0.7	26.2 ± 2.6	30.0 ± 1.9	12.9 ± 3.8	15.5 ± 3.5	0.7 ± 0.3
5	75	660	49.5	0.59 ± 0.13	0.8 ± 0.1	10.3 ± 0.7	2.6 ± 0.4	1.7	0.9 ± 0.4	71.3 ± 9.8	83.0 ± 9.4	17.8 ± 3.1	19.2 ± 3.6	0.6 ± 0.3
6	75	1800	135	0.47 ± 0.10	0.6 ± 0.1	12.5 ± 1	3.2 ± 0.2	1.0	2.2 ± 0.2	91.9 ± 16.8	106.6 ± 13.4	7.5 ± 1.5	9.5 ± 1.9	0.8 ± 0.4
7	150	420	63	0.99 ± 0.03	0.8 ± 0.1	9.9 ± 0.6	2.9 ± 0.6	1.3	1.6 ± 0.6	143.7 ± 24.2	166.9 ± 15.9	72.7 ± 9.0	84.1 ± 7.8	0.7 ± 0.1
(b)	Experiment 2 from 2013 [34]; individual ungrouped mice were used. Standard deviation of necrosis radius was taken as 0.5 mm for all cases													
8	12	4000	48	0.43	0.7	6.5	2.5	1.3	1.2	21.4	23.4	2.5	2.3	0.62
9	12	6000	72	0.17	0.7	11.2	3.1	2.3	0.8	13.9	16.1	0.6	0.8	0.25
10	20	3000	60	0.17	0.7	13.5	2.4	2.3	0.1	24.3	28.4	1.2	1.1	0.24
11	20	4000	80	0.32	0.7	15.2	3.0	1.3	1.7	23.8	27.9	1.9	2.2	0.48
12	30	1980	59.4	0.42	0.6	10.8	2.0	0.8	1.2	40.6	46.4	4.9	3.6	0.59
13	30	4500	135	0.35	0.5	14.3	1.4	0.8	0.6	44.1	51.0	2.1	0.9	0.57
14	75	300	22.5	0.25	0.7	12.2	2.6	2.3	0.4	137.3	155.4	15.3	16.1	0.25
15	150	180	27	0.17	0.5	9.8	2.8	2.3	0.5	203.4	231.3	15.7	17.3	0.15
16	150	660	99	0.17	0.8	9.4	2.0	2.3	0	147.5	170.5	6.5	4.6	0.25

^aLinear source strength.

^bTotal energy delivered per length.

Table 2

Photochemical parameters obtained for BPD.

Photochemical parameter	Initial value ^a	Fit value ^b
ϵ (cm ⁻¹ μ M ⁻¹)	0.0783	
δ (μ M)	33 [38]	
β (μ M)	11.9 [39]	
σ (μ M ⁻¹)	(0.5–10) $\times 10^{-5}$	(1.8 $\pm$ 3) $\times 10^{-5}$
ξ (cm ² mW ⁻¹ s ⁻¹)	(10–100) $\times 10^{-3}$	(55 $\pm$ 40) $\times 10^{-3}$
g (μ M s ⁻¹)	0.5–2.0	1.7 $\pm$ 0.7
[¹ O ₂] _{rx,sh} (mM)	0.5–2.0	0.67 $\pm$ 0.13
[³ O ₂] ₀ (μ M)	40 ^c [26]	

^aThe initial guess of parameters were assigned randomly within the presented ranges.

^bThe obtained values by the macroscopic model with their overall error. Note that the value of ξ is larger than previously reported in Ref. [34]. This is proportional to a change of ϵ from 0.034 to 0.078 based on experimental verification of the original literature value being based on log10 rather than loge as used in the current study. Each value is presented as the mean $\pm$ standard deviation.

^cThe initial ground state oxygen concentration was kept constant for all mice using a value from Ref. [26].