

The use of diffuse optical spectroscopy and diffuse correlation spectroscopy system for monitoring of tumor response to photodynamic therapy

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ABSTRACT

Photodynamic therapy (PDT) of cancer works via direct cytotoxicity, causing damage to tumor vasculature and stimulating the body's anti-tumor immune response. PDT outcome depends on the parameters used; therefore an in vivo tumor response monitoring system is useful for optimization of the treatment protocol. The combined use of diffuse optical spectroscopy and diffuse correlation spectroscopy allows us to measure the tissue oxygen saturation (StO₂) and relative blood flow (rBF) in tumors. These parameters were measured before and after PDT in mouse tumor models and were calculated as ratios relative to the baseline in each tumor (rStO₂ and rBF). Readings were also measured in drug-only control tumors. In responders (mice with tumor eradication), significant PDT-induced decreases in both rStO₂ and rBF levels were observed at 3h post-PDT. The rStO₂ and rBF readings in these mice remained low until 48h post-PDT, with recovery of these parameters to baseline values observed 2 weeks after PDT. In non-responders (mice with partial or no response), the rStO₂ and rBF levels decreased less sharply at 3h post-PDT, and the rBF values returned toward baseline values at 48h post-PDT. By comparison, the rStO₂ and rBF readings in drug-only control tumors showed only fluctuations about the baseline values. Thus tumor response can be predicted as early as 3h post-PDT. Recovery or sustained decreases in rStO₂ and rBF up till 48h post-PDT were correlated to long-term tumor control. Diffuse optical measurements can thus facilitate early assessment of tumor response to PDT to aid in treatment planning.

Keywords: Photodynamic therapy, response monitoring, diffuse optical spectroscopy, diffuse correlation spectroscopy, tissue oxygenation, relative blood flow

1. INTRODUCTION

Photodynamic therapy (PDT) is a promising cancer treatment modality that works through the interaction of a photosensitive drug, light and tissue oxygen to eradicate tumors [1]. The main mechanisms of PDT action are direct tumor cell killing, causing damage to tumor vasculature and stimulation of the body's anti-tumor immune response [2-3]. Conventional cancer treatment modalities have their limitations and PDT offers several advantages. Conventional therapies are subject to treatment limit while PDT can be safely repeated and administered in combination with other treatment modalities. While some cancer treatment can lead to immuno-suppression, PDT has potential to activate the body's anti-tumor immune response. Moreover, some patients fail conventional cancer treatments and may require alternative options. As PDT is being developed for clinical applications, there is a need to develop complementary techniques to evaluate treatment response to optimize the PDT regime. Since PDT works via interaction of a drug, light and tissue oxygen, the outcome depends on the treatment parameters used [4-6]. For each tumor type and drug used, the PDT parameters have to be optimized for best treatment outcome. Currently, tumor response to PDT is assessed by observation, tumor size measurement and/or histopathological examination in the clinic. There might be a delay between assessment and decision for further treatment. There is thus a need to develop a non-invasive tumor response monitoring system to provide early response assessment in the clinic. Optical spectroscopic techniques have been used for non-invasive clinical monitoring of tumor response to radiation therapy with promising results [7]. In this study, we present the combined use of diffuse optical spectroscopy and diffuse correlation spectroscopy to monitor tumor response to PDT.

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1.1 Diffuse Optical Spectroscopy

Diffuse optical spectroscopy (DOS) can be used to probe the optical properties, such as absorption and scattering coefficients, of biological tissue and this technique has been applied to treatment response monitoring [7]. When applied to PDT response monitoring, DOS can be used to measure the tissue oxygenated hemoglobin (HbO_2), deoxygenated hemoglobin (Hb), total hemoglobin concentration (THC), and oxygen saturation (StO_2) levels [8-9]. The StO_2 can be calculated as a percentage by using the equation below.

$$StO_2(\%) = \frac{[HbO_2]}{[HbO_2 + Hb]} \times 100\%$$

1.2 Diffuse Correlation Spectroscopy

In diffuse correlation spectroscopy (DCS), use of the autocorrelation function of fluctuating light intensities to calculate the average flow rate of scattering particles allows us to measure relative flow in deep tissue [10]. DCS can be used to measure the relative blood flow (rBF) in a tumor that has undergone PDT [11], thus allowing us to assess both the extent and time evolution of vascular damage caused by PDT, since PDT is known to work via causing damage to tumor vasculature.

1.3 Combined DOS and DCS Spectroscopy

The combined use of DOS and DCS allows us to measure HbO_2 , Hb, THC, StO_2 and rBF in tumors. Using our prototype DOS-DCS system, we measured these hemodynamic parameters in tumor relative to pre-treatment baseline values and correlated them to treatment outcome in an in vivo tumor model. These parameters are relevant for PDT response monitoring since PDT is an oxygen-consuming therapy regimen and it is known to work via causing damage to tumor vasculature.

2. MATERIALS AND METHODS

2.1 In vivo photodynamic therapy

Sub-cutaneous xenograft tumors of mouse mammary carcinoma (EMT-6) were induced in Balb/c mice. Tumors were subject to PDT when the tumors reached approximately 8 mm in diameter. The photosensitizer chlorin-e6 (Ce6) (ApoCare GmbH, Germany) was administered intravenously at a dose of 5 mg/kg. At 3h post drug administration, light at 665nm was delivered by a laser (Biolitec, Germany). The laser beam was expanded to illuminate a diameter of 2.5cm on the treatment surface. The light was delivered via an optical fiber at 85-130 mW/cm² for a light dose of 100-200 J/cm². The tumor StO_2 and rBF readings were measured in vivo before and after PDT. To account for variations in individual mice, the StO_2 and rBF readings measured after PDT were calculated as ratios relative to the baseline values in each mouse before Ce6 administration (r StO_2 and rBF). Mice were anesthetized during PDT and DOS and DCS measurements to minimize mouse movement. The tumor r StO_2 and rBF were measured up to 2 weeks post-PDT and correlated to tumor regression or re-growth. Tumor sizes were measured every 2 days. Mice that showed tumor response (eradication) post-PDT were defined as responders (n=3). Mice that showed partial response (with subsequent tumor re-growth) or no response post-PDT were defined as non-responders (n=6). The r StO_2 and rBF values were also measured in drug-only control tumors that received 5 mg/kg Ce6 but no light (n=7). These values were also calculated relative to baseline values before Ce6 administration. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Singapore Health Services.

2.2 DOS setup

For DOS, a frequency domain tissue oximeter (OxiplexTS, ISS Inc., USA) with a customized optical probe was used to deliver radio-frequency intensity-modulated light to the tumor at 2 wavelengths (690nm, 830nm) using 4 source-detector distances for each wavelength. Water concentration was assumed to be 75%. The contact optical probe was a fiber bundle consisting of 8 source fibers and 1 detector fiber. The tips of the illumination and detector fiber bundles in the probe are in alignment, with the source fibers at varying distances between 0.5mm to 4mm from the detector fiber. Figure 1 shows a photograph of the front panel of the DOS control module (left) and the use of a contact probe on a mouse model under inhalable anesthesia (right).



Figure 1. Photograph of the front panel of the DOS control module (left) and the use of a contact probe on a mouse model under inhalable anesthesia (right).

2.3 DCS setup

For DCS, a continuous-wave, long coherence length ($>10\text{m}$) laser operating at 785 nm (CrystaLaser, USA) was coupled into a multi-mode optical fiber with $400\mu\text{m}$ core diameter, illuminating scattering particles of tumor tissue by contact. A single-mode fiber gathers photons 8mm away from a single speckle emitted from the tumor surface. Light intensity fluctuations were detected by a photon counting avalanche photodiode (APD) (Perkin-Elmer, Canada). The output of the APD was a stream of transistor-transistor logic (TTL) pulses, which were fed to a 32-bit, eight input channels counter/timer board through a shielded I/O connector block for data acquisition (DAQ) devices, SCB-68 (National Instruments, USA), to be counted. Figure 2 shows a photograph of the DCS set-up. The absorption and scattering properties, μ_a and μ_s' of the tumor tissue measured by DOS at 830 nm were used as fixed parameters. The relative blood flow, parameterized by the blood flow index, $\text{BFI} = \alpha D_B$, where α is the volume fraction of moving scatterers out of all scatterers, and D_B is the effective diffusion coefficient of scatterers, was obtained by fitting the data with a Brownian motion model. In this study, we define rBF as $\text{BFI} / \text{BFI}_{\text{baseline}}$.

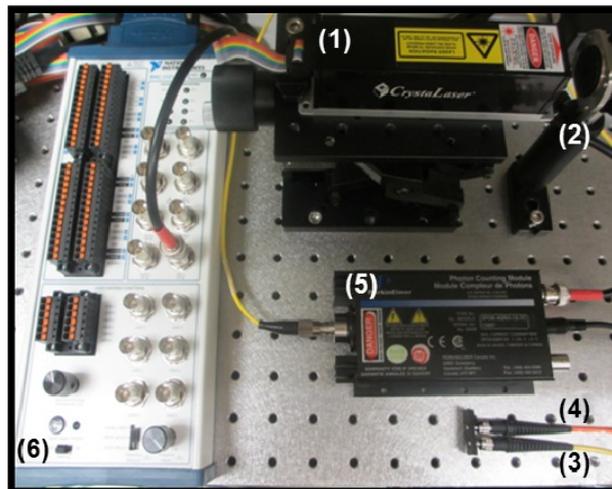


Figure 2. Photograph of the DCS set-up consisting of (1) a 785nm laser, (2) collimator, (3) source fiber, (4) detector fiber, (5) photon counting module and (6) a shielded I/O connector block.

3. RESULTS

Mice models bearing xenograft tumors were subjected to Ce6-PDT as described and treatment response was assessed using DOS and DCS to measure the tumor oxygen saturation (StO₂) and relative blood flow (rBF). Figure 3 shows the mean tumor rStO₂ and rBF values in responders (n=3) expressed as ratios of the baseline readings measured before Ce6 administration at “-3h”, 3h prior to PDT at “0h”. Responders (mice with tumor eradication) showed significant PDT-induced decreases in both the mean rStO₂ and rBF levels at 3h post-PDT. The mean rStO₂ and rBF readings in these mice remained low up till 48h post PDT, implying damage to the tumor vasculature. Recovery of these parameters to baseline values was observed 2 weeks after PDT. In non-responders (mice with only partial or no tumor response), the mean rStO₂ and rBF levels also decreased at 3h post-PDT, but less sharply (Figure 4). Moreover, the rBF in non-responders returned toward baseline values at 48h post-PDT, implying tumor vasculature repair or angiogenesis (Figure 4(b)). By comparison, the mean rStO₂ and rBF readings in drug-only control tumors showed only fluctuations about the baseline values following Ce6 administration at “-3h” (Figure 5). Figure 6 shows the images of a treated tumor from the responder group, showing the tumor on the day of PDT, and at 48h, 1 week and 2 weeks post-PDT. The tumor was eradicated and long-term follow up subsequently showed no relapse of the tumor up to 9 months post-PDT.

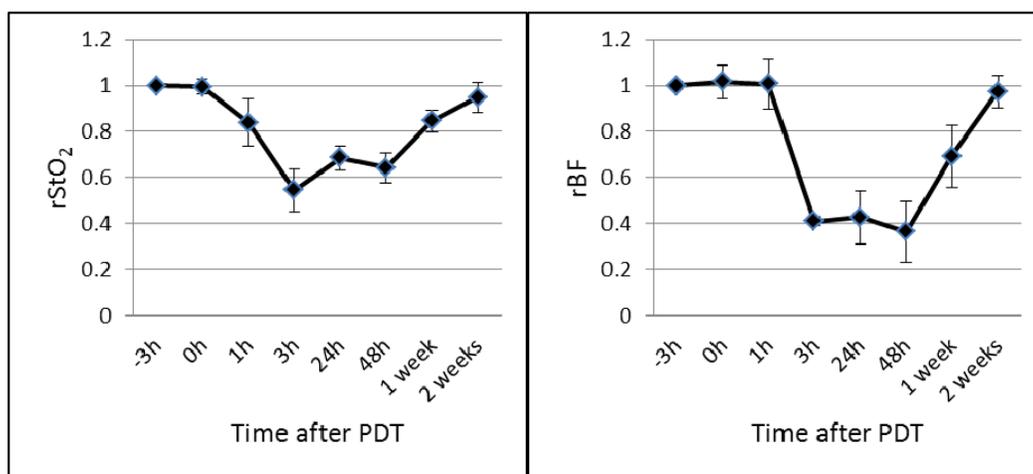


Figure 3. Mean tumor rStO₂ and rBF values in responders (n=3) expressed as ratios of the baseline readings measured before Ce6 administration at “-3h”, 3h prior to PDT at “0h”. The standard errors of the mean are presented as error bars.

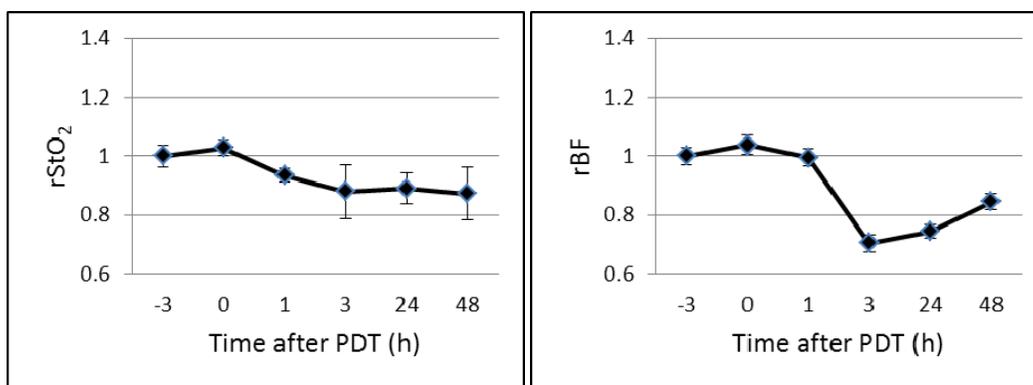


Figure 4. Mean tumor rStO₂ and rBF values in non-responders (n=6) expressed as ratios of the baseline readings measured before Ce6 administration at “-3h”, 3h prior to PDT at “0h”. The standard errors of the mean are presented as error bars.

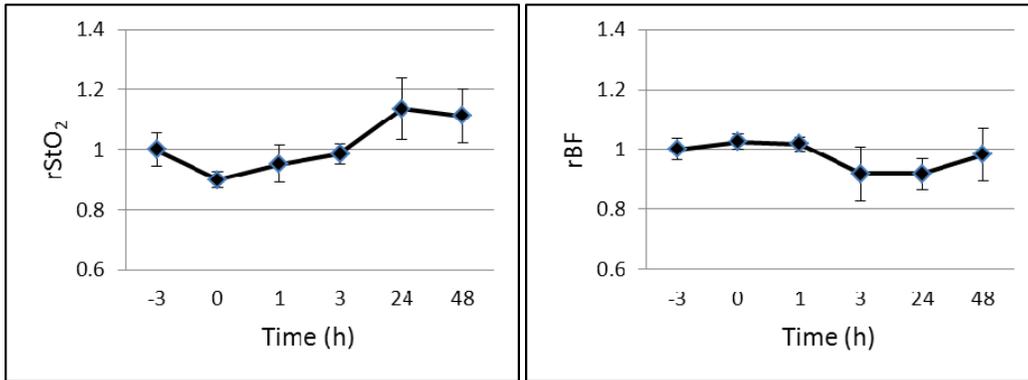


Figure 5. Mean tumor rStO₂ and rBF values in drug-only control tumors (n=7) expressed as ratios of the baseline readings measured before Ce6 administration at “-3h”. The standard errors of the mean are presented as error bars.

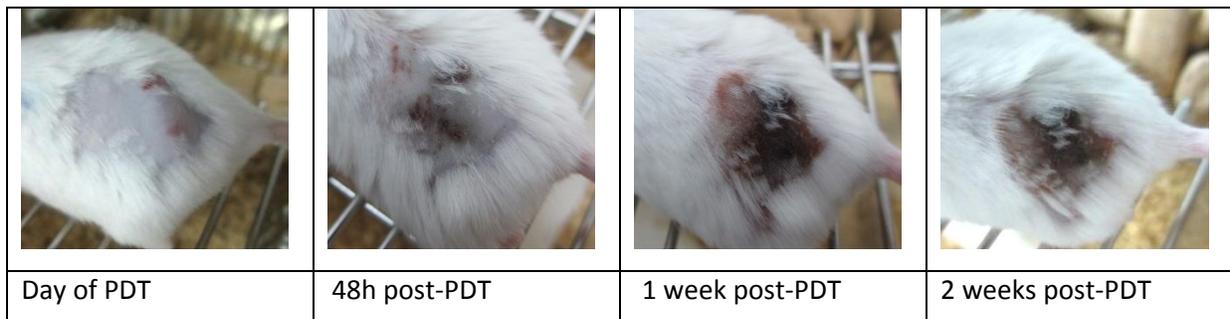


Figure 6. Images of a treated tumor from the responder group showing the tumor on the day of PDT, and at 48h, 1 week and 2 weeks post-PDT. The tumor was eradicated and long-term follow up subsequently showed no relapse of the tumor up to 9 months post-PDT.

4. DISCUSSION AND CONCLUSIONS

We have set up a combined DOS and DCS system to assess tumor response to PDT by measuring the tumor oxygen saturation levels and blood flow relative to pre-treatment baseline in individual tumors (rStO₂ and rBF). The rStO₂ and rBF levels exhibited distinctly different patterns of treatment-induced variations in responders (mice with tumor eradication) versus non-responders (mice with partial or no response) within the first 48 hours post-PDT. A large PDT-induced decrease in these parameters at 3h post-PDT was indicative of a better response and a sustained decrease in these parameters up till 48h and beyond correlated with long-term tumor eradication.

Overall, the results from this study show that tumor response to PDT can be predicted by assessing variations in the tumor rStO₂ and rBF levels as early as 3h post-PDT. Moreover, recovery or sustained decreases in these hemodynamic parameters up till 48h post-PDT correlated to long-term tumor control. Work is underway to further develop a system with real-time data analysis capabilities to better facilitate same-day treatment response assessment in a clinical setting. Our long-term aim is to develop diffuse optical spectroscopy for non-invasive early assessment of tumor response to PDT in a clinical setting as an aid to treatment planning.

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