Non-invasive monitoring hemodynamic responses in RIF tumors during and after PDT

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ABSTRACT

Changes in blood flow and oxygenation during and after PDT provide information about tumor vessel and cellular damage. The characterization of these changes may improve our understanding of PDT mechanisms and help predict treatment efficacy. We have designed a hybrid system that can non-invasively measure *in vivo* hemodynamic changes and provide independent information about tumor oxygenation and blood flow. Diffuse correlation spectroscopy (DCS) monitors blood flow by measuring the optical phase shifts caused by moving blood cells, while diffuse photon density wave (DPDW) spectroscopy measures tissue absorption and scattering. When mounted on a camera, our unique probe allows non-contact measurements that avoid compressing the tumor and altering blood flow. An optical filter mounted in front of the camera lens cut off light below 650nm, which allowed monitoring of blood flow during PDT. The utility of the hybrid system was demonstrated by monitoring the hemodynamic changes during and after PDT in mice bearing the experimental radiation-induced fibrosarcoma (RIF). For the first time, we non-invasively and continually monitored the *in vivo* flow changes during PDT. Relative oxygen consumption was calculated using flow values measured by DCS and oxygenation measured by a broadband absorption spectrometer. During PDT an initial rapid increase in blood flow was found, followed by a decrease and slow recovery. After PDT, substantial and continued reductions in blood saturation, blood flow and oxygen consumption were found after 3 hours, suggesting that permanent damage to tumor cells and blood vessels had occurred. The comparison of flow values after PDT as measured by DCS and by Power Doppler ultrasound (CWFA) demonstrated that both techniques non-invasively detected similar global changes in tumor blood flow or perfusion after PDT.

Keywords: PDT, hemodynamics, blood vessel, diffuse photon density wave (DPDW) spectroscopy, diffuse correlation spectroscopy (DCS), non-contact probe, power broadband absorption spectrometer, Doppler ultrasound

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1. INTRODUCTION

Photodynamic therapy (PDT) is a relatively new experimental cancer therapy that has been used with some success for the treatment of solid tumors and surface malignancies. The method requires administration of a photosensitizer that localizes in tumor tissue and is subsequently activated by exposure to optical radiation. The excited photosensitizer initiates a cascade of chemical reactions, often involving highly reactive oxygen intermediates that can cause selective necrosis and apoptosis of cells as well as vascular damage. The damage is mainly due to the formation of singlet oxygen resulting from the interaction of light excited photosensitizer with molecular oxygen (triplet ground state). Such damage to tumor capillaries may induce regional tissue hypoxia during PDT and thus be a barrier to effective treatment[1]. Monitoring of the *in vivo* hemodynamic changes within tumors during PDT may reveal the extent of cellular and vascular damage. Studies designed to quantify the photochemical oxygen consumption and blood flow during and after PDT may improve our understanding of PDT mechanisms and lead to improved therapeutic strategies.

There are a number of techniques for measuring the oxygenation of tumors. Polarographic needle electrodes (pO2 Histograph) enable macroscopic, invasive measurements of tissue pO2[2]. O2 microelectrodes can sample tissue volumes that are much smaller than the distance between capillaries and hence can provide information on how PDT treatment affects oxygen transport at the microregional level[2-4]. However, the invasive nature and poor spatial resolution of both of these techniques limits their application in humans. Near-infrared spectroscopy (NIRS) uses light in the 600-900nm range permitting noninvasive measurements although with reduced spatial resolution due to the scattering of light in the tissue[5]. This technique permits the measurement of hemoglobin oxygen saturation at distances of up to several centimeters deep tissue.

Laser Doppler can monitor non-invasively the flow change only in tissue surface (penetration depth < 500 μm). Pogue et al. invasively monitored flow changes during PDT with laser Doppler using fiber probes inserted into the tumor[4]. Their results indicated that the irradiation light saturates the Doppler probe so that the only useful data are between light fractions. Power Doppler ultrasound can non-invasively follow changes in tumor perfusion after PDT through determination of the color-weighted fractional average (CWFA) in acquired images. However, it can't be used during PDT.

It is more difficult to develop reliable techniques for *in vivo* detection of hemodynamic changes containing independent information about hemoglobin concentration, oxygenation and flow. Pogue et al. has employed laser Doppler (flow) and O2 microelectrodes (pO2) to invasively study the heterogeneity of pO2 dynamics during PDT with Verteporfin[4]. A goal of our work is to design a NIRS system that can non-invasively measure the hemodynamic changes containing independent information about oxygenation and flow. In this study, we built a novel hybrid instrument that combines two NIR diffuse optical techniques: Diffuse Photon Density Wave spectroscopy (DPDW) for blood oxygenation measurement and Diffuse Correlation Spectroscopy (DCS) for relative blood flow measurement. Mounted on the back of a camera, our unique probe allows non-contact measurements that avoid compressing the tumor and altering blood flow. An optical filter mounted in front of the camera lens cut off light below 650nm, which allows the monitoring of blood flow even when the PDT-exciting light (630 nm) is on because the wavelengths used for DPDW and DCS are in the range of 690-830 nm. The utility of this system is demonstrated by monitoring hemodynamic changes during and after PDT. The flow measurements obtained by DCS have the same trend as those measured by Power Doppler ultrasound. To quantify the optical properties of the tumor, a broadband absorption spectrometer was employed to simultaneously measure the wavelength-dependent reflectance of tissue at many source-detector separations before and after PDT. Those values were also used as input parameters for DCS and DPDW analysis.

2. MATERIALS AND METHODS

2.1 Tumor model and PDT

RIF (radiation-induced fibrosarcoma tumors) were propagated on the shoulders of C3H mice (Taconic, Germantown, NY) by the intradermal injection of 3 x 10^5 cells. Animals were treated ~1 week later when tumors were about 6 mm in diameter. The photosensitizer Photofrin (Axcan Pharma Inc., Mont-Saint-Hilaire, Quebec) was administered via tail vein at 5 mg/kg at ~24 h before illumination. The laser system consisted of a KTP YAG pumped

dye module (Laserscope, San Jose, CA) tuned to produce 630 nm light. Light was delivered to a 1 cm diameter treatment field through microlens-tipped fibers (CardioFocus, Norton MA) and the power density was measured with a power meter (Coherent, Auburn, CA). Treatment was to a total fluence of 135 J/cm^2, delivered at 75 mW/cm^2. Mice were divided into two groups: treated group = tumor + drug + light and control group = tumor + light. During PDT and the optical measurements the mice were anesthetized with isoflurane and kept warm on a heating pad.

2.2 The hybrid system for oxygen and flow measurement combining DPDW Spectroscopy and DCS Spectroscopy

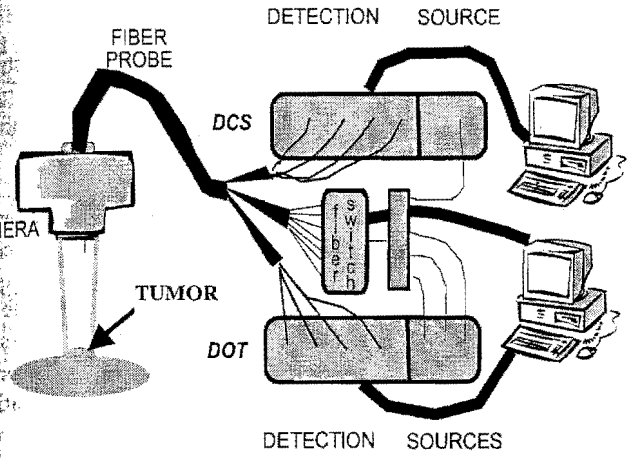


Figure 1. The scheme of the hybrid instrument for measuring the DPDW and DCS in mouse tumor.

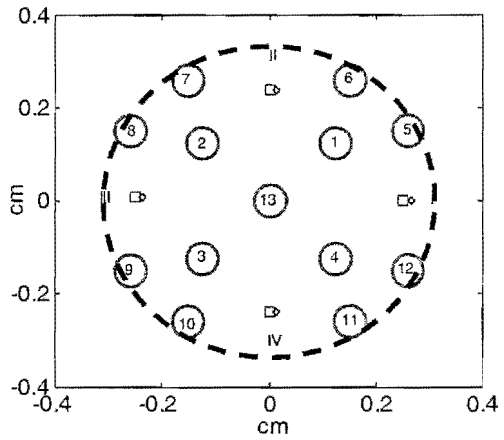


Figure 2. The optical fibers for 13 source positions (small circle), four DCS detectors (diamond) and four DPDW detectors (square) are arranged in a two-dimensional pattern. The big dashed circle presents the contour of the tumor.

The basic optical unit is the hybrid instrument that is described in detail in a companion paper in the same volume by Yu et al[6]. Figure 1 shows a schematic diagram of the instrument. Three laser diodes (690nm, 786nm, 830nm) modulated in 70MHz were employed in frequency domain DPDW measurements, while one single mode laser (800nm) operating in continuous mode was used as the source for DCS measurement. Several optical switches (switch and setup time > 350ms) were employed to switch the four wavelengths and 13 source positions consecutively. Two sets of four avalanche photodiodes were employed for detection of DPDW and DCS separately. The two computers working as two control panels for DPDW and DCS communicated through two digital I/O lines.

A non-contact probe with 13 source fibers, four detector fibers for DPDW, and four detector fibers for DCS, was used to avoid potentially compressing the tumor and altering blood flow. One optical filter mounted in front of the camera lens cut off light below 650nm, which allows monitoring blood flow during PDT. The optical fibers for the DPDW sources, the DCS sources, the DPDW detectors, and the DCS detectors, were arranged in a two-dimensional pattern shown in figure 2. The probe covered 6×6 mm^2 area. The fibers were hold by a camera back whose lens was 15 cm from the tumor. With this probe, the hybrid instrument required 18 seconds to scan one frame including 13 source positions, 4 wavelengths, and 4 detectors for DPDW and four detectors for DCS.

The DPDW amplitudes and phases are then analyzed to extract bulk optical properties (μ_s' and μ_a) or their spatial distribution. Here μ_a is the absorption coefficient and μ_s' is the scattering coefficient. Use of multiple wavelengths and the extinction coefficient spectra of tissue chromospheres such as oxy- and deoxy- hemoglobin, enables us to compute blood oxygen saturation and blood volume. Changes in blood flow within turbid media can be determined from measurements of the temporal intensity autocorrelation functions of the diffused light[7]. In particular, time (t) dependence of the autocorrelation functions has the form ~ $e^{-t/\tau}$. The temporal decay rate, ~$(1/\tau)$, is proportional to the mean square blood flow speed within the measured volume. From the normalized intensity autocorrelation

function, we calculate the diffuse electric field temporal autocorrelation function, which satisfies the correlation diffusion equation. The diffuse light electric field autocorrelation function is analyzed using the Siegert relation. The correlation function decays according to decay constant $k_D^2 = 3\mu_s'\mu_a + 6\mu_s'^2\mu_a^2 k_o^2 \alpha D_B \tau$, where k_0 is the wavenumber of the light in the medium. The probability that a photon is scattered by a moving "cell", α, is presumed proportional to blood volume. The blood flow speed is parameterized by a Brownian diffusion constant D_B and the product αD_B parameterizes blood flow. The measurements of G can be fitted to yield αD_B. The absorption coefficient μ_a and the scattering coefficient μ_s' were obtained by our broad absorption spectrometer. Detail information about these approximations could be found in previous papers[6, 8, 9].

2.3 Broadband absorption spectrometer

The primary drawback of the hybrid system is that it is not a broadband spectral device. In our PDT studies, only few source-detector separations yield information about the tissue properties due to the small volume of the tumor. It is difficult to get absolute values of the properties with limited source-detector pairs and limited number of wavelengths.

A broadband absorption spectrometer has been built to simultaneously measure the wavelength-dependent reflectance of tissue at many source-detector separations. The system consists of four major parts: the light source, the fiber-optics probe head, the CCD camera, and the dispersion system (monochromator). Light from a 250 W quartz tungsten halogen lamp is coupled to the tissue surface through a source fiber and detected in the reflectance mode from the tissue surface by 10 detection fibers. These fibers are arranged in a line and have source-detector separation distance ρ at 0.6, 1.2, 1.8, 2.4, 3, 4, 5, 6, 8, and 10 mm. The collection fibers are then coupled back to the monochromator entrance slit where the ends of the detector fibers are arranged in a vertical line with equal space and well positioned relative to the monochromator such that the image of the fiber tips is projected and focus on the liquid nitrogen cooled CCD camera sensor. The grating disperses this light so that the image plan of monochromator output contains ten vertically spaced bright lines corresponding to the spectra, $R_{tissue}(\rho, \lambda)$ of ten detection fibers. The reflectance spectra $R_{tissue}(\rho, \lambda)$ can be used to recover tissue optical properties. A physical model to make quantification of these values is described in detail in a companion paper in the same volume by Wang et al[10].

2.4 Metabolic rate of oxygen consumption

We used a traditional formula to calculate the relative changes in oxygen metabolic rate of oxygen consumption[8, 9, 11]:

$$rOC = rOEF \times rBF \tag{1}$$

Here rOEF and rBF are oxygen extraction fractions and blood flow changes normalized to their baselines respectively.

$$OEF = (SaO2 - SvO2)/SaO2 = (SaO2 - StO2)/(\gamma \times SaO2) \tag{2}$$

Here SaO2 is arterial oxygen saturation that is assumed to be 98% and is nearly constant. StO2 is tissue oxygen saturation obtained by our measurements. SvO2 is the venous oxygen saturation. The γ indicates percentage of blood volume contained in the venous component of the vascular system and assumed to be constant, then

$$rOEF = 1 + \Delta OEF/OEF_0 = (SaO2 - StO2)/(SaO2 - StO2_0) \tag{3}$$

$$rBF = 1 + \Delta BF/BF_0 = BF/BF_0 \tag{4}$$

BF is the blood flow values measured by DCS. The corresponding baseline values are denoted by subscript 0.

2.5 Power Doppler ultrasound

Doppler ultrasound has been used extensively to assess vascularity in a range of animal and human tumors. In particular, power mode Doppler ultrasound appears to be a more sensitive and specific method for measuring tumor blood flow than traditional Doppler ultrasound[12]. Ultrasound imaging was performed on mice anesthetized i.e. with ketamine/ xylazine (150/10 mg/kg) and kept warm on a heating pad. Approximately 10 images were acquired of each tumor at each timepoint studied. From each image the Color Weighted Fractional Area (CWFA) was determined as a measure of perfusion, and values were averaged for each mouse at each timepoint.

3. RESULTS AND DISSCUSIONS

The hybrid instrument monitored continuously the oxygen and flow changes during PDT (from 15 minutes pre-PDT to 10 minutes after PDT). Additional measurements were performed at timepoints after PDT (3 hours, 6.5 hours and 24 hours after PDT). For the quantitative evaluation of tissue optical properties broadband absorption spectrometric measurements were made at several time points: 15 minutes before PDT, 15 minutes, 3 hours, 6.5 hours and 24 hours after PDT. The normalized percent changes from pre-PDT values were calculated. The flow measurement results by DCS were compared with CWFA as found by Power Doppler ultrasound at 15 minutes, 3 hours and 6.5 hours after PDT.

3.1 Flow measurement by hybrid system during and after PDT

We monitored the flow changes during PDT by DCS technique in seven treated mice and two control mice. A typical curve of flow changes during and after PDT in a treated mouse is shown in figure 3. The flow value at each time point is given in percent change relative to the baseline value (before PDT) and the error bar presents the standard error of the mean flow value from the four source-detector pairs shown in figure 2 (source no. 13 and four detectors for DCS).

After 15 minutes of pre-PDT measurements, the laser was turned on. Blood flow increased rapidly in a short time to its maximum value, and then decreased slowly. During PDT (30 minutes), the flow value reached its minimum value after 6-25 minutes from the start of treatment, and then it slowly increased. The flow increase continued after illumination ceased. We observed a similar trend in all seven treated tumors during PDT although the maximum value, minimum value, and time to reach the maximum or minimum did vary slightly. In contrast, in the control mice, only the initial rapid increase of the flow was found during PDT. Figure 4 shows the relative flow changes in a control mouse without Photofrin. Substantial reductions in blood flow and StO2 (see section 3.2) appeared in the drug-administrated mice, but not in the control mice, at 3 hours, 6.5 hours, and 24 hours after PDT. The flow reductions after PDT are compared with power Doppler ultrasound in section 3.3.

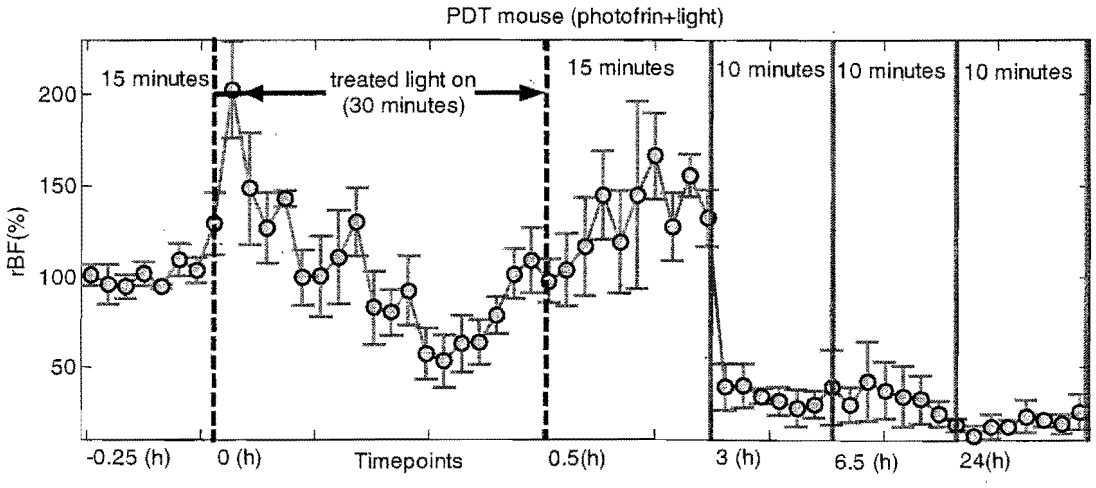


Figure 3. Flow changes measured by DCS before, during and after PDT in a treated mouse.

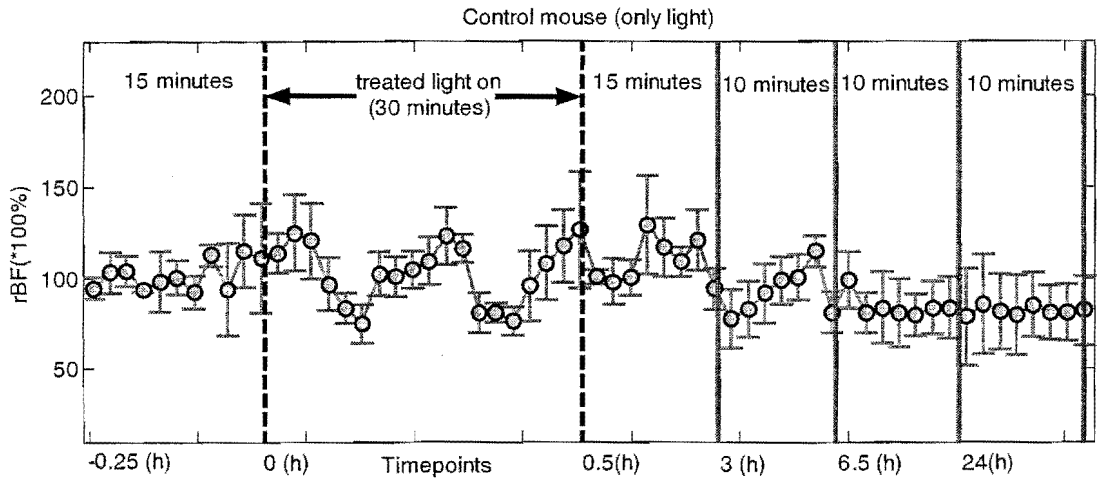


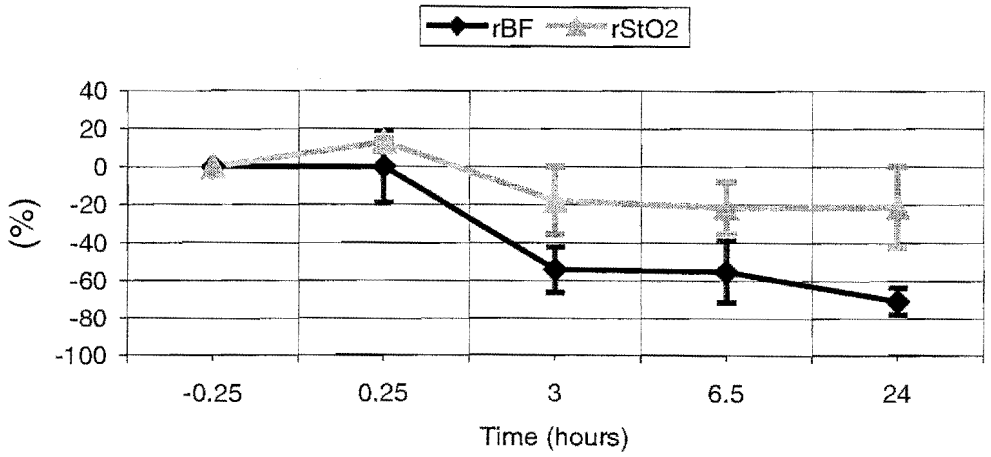
Figure 4. Flow changes measured by DCS before, during and after illumination in a control mouse.

The rapid increase in flow found immediately after beginning illumination may be attributed to a heating effect since this trend was found in both the control and the PDT treated animals. Pogue et al. observed a 1.4°C maximum change of temperature in rat tumors after 2 min of light irradiation (200mW/cm^2 at 690nm)[4]. In the PDT-treated animal, following the rapid increase in flow, a decrease to values below baseline occurred. It appears that this slow decrease indicates temporary vessel restriction or occlusion during PDT since a recovery in flow began before PDT concluded. Using laser Doppler a similar flow trend during PDT was found by Pogue et al. although a different animal model was studied[4]. The unrecoverable flow reduction that appeared in treated mouse tumors by 3 hours after PDT suggests that permanent vessel restriction or occlusion had occurred.

3.2 Tumor oxygen consumption

We used a broadband absorption spectrometer to quantify the tissue properties and oxygenation at several time points: 15 minutes before PDT, 15 minutes, 3 hours, 6.5 hours and 24 hours after PDT. Oxygen consumption can be calculated using these values and the relative flow values obtained by DCS according the formula (1).

Figure 5 shows the relative change in tissue oxygen saturation (rStO2), blood flow (rBF) and oxygen consumption (rOC) in four treated mice. All values at each time point are given in percent change relative to the baseline value (15 minutes before PDT) and the error bar shows the standard error.



(a)

The flow measurement results by DCS were compared with the Power Doppler ultrasound at 15 minutes, 3 hours and 6.5 hours after PDT. These results are presented in Figure 6. Values at each time point are given in average percent change relative to the baseline value (15 minutes before PDT) and the error bars indicate the standard error from four (DCS) or five (CWFA) tumors. We observed substantial flow reductions at 3 and 6.5 hours after PDT as measured by both techniques. Insignificant changes in flow were found after tumor illumination in the absence of photosensitizer. Good agreement was found between the two methods and the small differences may be attributed to the fact that different groups of mice were followed for the DCS and the ultrasound studies because of technical limitations.

4. SUMMARY

Monitoring of the *in vivo* hemodynamic changes within tumors during and after PDT may provide information on the extent of cellular and vascular damage and lead to improved therapeutic strategies. We have designed a hybrid system that can non-invasively measure *in vivo* hemodynamic changes and provide independent information about tumor oxygenation and blood flow. For the first time, the flow changes during PDT were monitored non-invasively and continually by diffuse correlation spectroscopy (DCS). After PDT, the substantial and continued reductions in blood saturation, blood flow and oxygen consumption suggested that permanent damage to tumor cells and blood vessels had occurred. We compared the flow values obtained by DCS with tumor perfusion as determined by the CWFA from power Doppler ultrasound images. The good agreement between these methods validates our technology in development.

ACKNOWLEDGMENTS

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