Interlesion differences in the local photodynamic therapy response of oral cavity lesions assessed by diffuse optical spectroscopies

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Abstract: Photodynamic therapy (PDT) efficacy depends on the local dose deposited in the lesion as well as oxygen availability in the lesion. We report significant interlesion differences between two patients with oral lesions treated with the same drug dose and similar light dose of 2-[hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH)-mediated photodynamic therapy (PDT). Pre-PDT and PDT-induced changes in hemodynamic parameters and HPPH photosensitizer content, quantified by diffuse optical methods, demonstrated substantial differences between the two lesions. The differences in PDT action determined by the oxidative cross-linking of signal transducer and activator of transcription 3 (STAT3), a molecular measure of accumulated local PDT photoreaction, also showed >100-fold difference between the lesions, greatly exceeding what would be expected from the slight difference in light dose. Our results suggest diffuse optical spectroscopies can provide in vivo metrics that are indicative of local PDT dose in oral lesions.

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1. Introduction

Photodynamic therapy (PDT) is an emerging treatment option for head and neck cancer [1]. It has been successfully applied for lesions in the oral cavity with a high degree of preservation of vital organ functions such as speech and swallowing [1,2]. PDT efficacy depends on the deposited local dose, which is proportional to administrated drug (photosensitizer) dose and light dose. Light dose typically is the prescribed administered dose and is measured as fluence. The optical properties of the lesions can affect the light absorption and scattering resulting in variations in the deposited light dose. Photosensitizer dose can also show substantial variations [3–5] due to physiological differences determining uptake and retention. For example, high blood flow can allow more efficient drug (photosensitizer) delivery and distribution in tissue. PDT is a dynamic process and PDT-related vascular parameters such as blood flow and oxygenation can change during PDT. Higher blood flow and oxygenation during PDT correlate with improved PDT efficacy [6,7]. Similarly, photobleaching of the photosensitizer via the generation of singlet oxygen (\(1{\text{O}}_2\)) during the photodynamic process is related to PDT efficacy [8,9]. Thus, in addition to pre-PDT values, PDT-induced changes in hemodynamic parameters and photosensitizer content can serve as indicators of PDT response [3].

In our previous work, we demonstrated the feasibility of a combined instrument that utilized diffuse correlation, diffuse reflectance and diffuse fluorescence spectroscopies in monitoring changes induced by 2-[hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH)-mediated PDT in oral lesions [10]. In this communication, we report an interesting case where two patients had lesions treated with the same administrated photosensitizer dose and a similar delivered light dose, but the absorbed doses, as determined by the oxidative cross-linking of signal transducer and activator of transcription 3 (STAT3) revealed > 100-fold differences. STAT3 crosslinking has been shown to be an internal molecular measure for the cumulative photoreaction [11,12]. We quantified local PDT-related parameters such as photosensitizer content, blood flow, blood oxygen saturation and blood volume with diffuse optical methods to investigate whether this substantial difference could be detected noninvasively. The results showed significant differences in HPPH concentration, blood flow and blood oxygen saturation between these patients at pre-PDT. Moreover, the photoreaction induced changes in HPPH content, blood flow and blood volume were significantly different. These results indicate that parameters quantified with diffuse optical spectroscopies at pre-PDT as well as PDT-induced changes may be indicative of local PDT reaction within individual lesions and may assist in predicting PDT outcome.

2. Materials and methods

2.1 Patient characteristics and measurement protocol

The current report was generated from a Phase-I clinical trial of HPPH-mediated PDT for patients with oral lesions, the main objective of which was to determine the maximum tolerated light dose at a fixed drug dose of 4.0 mg/m² [10]. The patient treatment and
measurement protocol was approved by the RPCI Institutional Review Board. The first patient (Patient-1) had a large carcinoma in situ (CIS) of the hard palate on the roof of the mouth and the second patient (Patient-2) had high grade dysplasia in a papilloma of the buccal mucosa (Fig. 1). For both patients, HPPH was administered intravenously (IV) 24 hours before optical measurements and PDT treatment, the light fluence rate was 150 mW/cm² and the treatment wavelength was 665 nm, corresponding to the in vivo absorption peak of HPPH. The first patient was in the cohort that received 125 J/cm² and the second patient was in the cohort that received 140 J/cm². The light source for the PDT treatment was a Coherent dye laser pumped by an Argon ion laser (Spectra Physics), and the light was delivered by a single quartz lens fiber. The treatment beam was centered on the lesion with the beam diameter slightly larger than the lesion diameter such that the periphery of the lesion was also within the treatment field. The size of the treatment field was 3.5 cm for Patient-1 and 2.5 cm for Patient-2. For each patient, optical measurements were acquired from within the lesion (identified by the surgeon) and the surrounding periphery of the lesion. There are two time points in the measurements: pre-PDT (baseline) and post-PDT (just after the end of PDT). At each time point, five measurements were acquired within the lesion and three measurements from the periphery. Multiple measurements were obtained by positioning the hand-held probe at a slightly different location.

2.2 Diffuse optical spectroscopies

We utilized diffuse correlation, reflectance and fluorescence spectroscopies for quantification of PDT-related parameters. The details of the instrumentation and quantification models were described recently [10]. Briefly, the diffuse correlation spectroscopy (DCS) [13–18] instrument consisted of a 785 nm, long coherence length laser, four photon-counting detectors and an autocorrelator board. After the DCS measurements were acquired, a second measurement system was used to collect diffuse fluorescence spectroscopy (DFS) and diffuse reflectance spectroscopy (DRS) data with a two-channel spectrometer incorporating a line CCD. The fluorescence excitation wavelength for HPPH was 410 nm and the power of the laser diode was 4 mW, low enough not to cause significant photobleaching. For reflectance measurements, the source was a tungsten halogen lamp. The source-detector separation was 0.8 mm for fluorescence measurements and 1.6 mm for reflectance and DCS measurements. A single hand-held probe contained all the source and detector fibers. All three spectroscopy systems were assembled on a mobile cart for performing measurements in the operating room.

For DCS data analysis, from the measured normalized temporal intensity autocorrelation function, one extracts the normalized electric field autocorrelation function, $g_1(r, \tau) = G_1(r, \tau)/\langle I \rangle$, where $G_1(r, \tau)$ is the electric field autocorrelation function, $\langle I \rangle$ is the time-averaged diffuse light intensity, $r$ is the source-detector separation and $\tau$ is the time.
delay. \( G_\tau(r,\tau) \) satisfies the correlation diffusion equation and the analytical solutions of the diffusion equation are available for simple geometries like reflectance geometry \[15,19\]. The decay rate of the autocorrelation function is related to optical properties and the mean square displacement \( \langle \Delta r^2(\tau) \rangle \) of moving scatterers such as blood cells. It has been observed in many physiological settings that a diffusion model, i.e., \( \langle \Delta r^2(\tau) \rangle = 6D_\tau \) provides better fits to the autocorrelation function and \( \alpha D_\tau \) characterizes blood flow related parameter \[20–22\], where \( D_\tau \) is an effective diffusion coefficient of moving scatterers and \( \alpha \) represents the fraction of scattering events originating from moving blood cells. In this paper, we report a blood flow index \( BFI \), as the ratio of the blood flow parameter of measured tissue, \( \alpha D_\text{tissue} \), to that of a reference Intralipid sample, \( D_\text{Intralipid} \) to compare the baseline contrast. Representative autocorrelation curves and fits for each patient (decay rate is related to blood flow) are shown in Fig. 2(a).

For DRS data analysis, a multi-wavelength fitting algorithm was applied to extract parameters \[23–25\]. The background counts were subtracted from the tissue reflectance before normalization with a diffuse reflectance standard. The spectral data were smoothed by binning every four pixels (corresponding to \( \sim 1.4 \) nm) out of 2048 pixels of line CCD and the mean and standard deviation for each bin were calculated. A Levenberg-Marquardt algorithm (lsqnonlin, Matlab) was used to fit the normalized measured data \( R_\text{data} \) to the calculated
reflectance ($R_i$) determined from an analytic diffuse reflectance model [26], by minimizing the chi-square error, $\chi^2 = \sum_{i=1}^{N} ((R_{data}(i) - R_{i}) / \sigma(i))^2$, where $\sigma(i)$ is the standard deviation of each binned data [27]. This model is valid when tissue scattering is much higher than tissue absorption and when the source detector separation is longer than several mean free paths of photons in the medium. In the wavelength range of interest, between 520 and 820 nm, this approximation typically holds accurate for a source detector separation of 1.6 mm. More improved approaches have been demonstrated such as P$_3$ approximation [28], Monte Carlo [29] and empirical [25] models for cases of higher absorption or shorter separations. The wavelength range used for fitting was 520–820 nm; thus $N = 211$ binned data were fitted simultaneously. Tissue scattering was assumed to follow Mie-type behavior, $\mu_s = a\lambda^{-b}$, where $a$ and $b$ are related to the size and concentration of scatterers [30,31]. Tissue absorption was assumed to be a linear combination of oxygenated, deoxygenated hemoglobin and HPPH photosensitizer absorption with fixed background absorption of water: $\mu_a = BVf(S,O_a\mu_o^{oxy} + (1-S,O_a)\mu_a^{deoxy} + C_{HPPH}\mu_a^{HPPH} + wf\mu_a^{water}$, where $BVf$ is the blood volume fraction, $S,O_a$ is the blood oxygen saturation, $C_{HPPH}$ is the HPPH concentration, $wf$ is the water fraction (fixed at 0.70), and $\mu_o^{oxy}$, $\mu_a^{deoxy}$, $\mu_a^{HPPH}$, $\mu_a^{water}$ are the absorption coefficients of oxy-hemoglobin, deoxy-hemoglobin, HPPH and water, respectively. Representative reflectance measurements from the lesion of each patient are shown in Fig. 2(b).

For DFS data analysis, background subtracted fluorescence signal was normalized with the reflectance data [32]. Every four pixels (corresponding to 1.35mm) were binned and the mean and standard deviation for each bin were calculated. Fluorescence signal was assumed to be a linear combination of HPPH, tissue autofluorescence (Auto), and components of the heme pathway such as protoporphyrin IX (PpIX), precursors coproporphyrinogen and uroporphyrinogen (CpUp) and a photoproduct of PpIX (Photo) [33]: $F_{tissue} = C_{HPPH}F_{HPPH} + C_{Auto}F_{Auto} + C_{PpIX}F_{PpIX} + C_{CpUp}F_{CpUp} + C_{Photo}F_{Photo}$, where $C_{HPPH}$, $C_{Auto}$, $C_{PpIX}$, $C_{CpUp}$, and $C_{Photo}$ are spectral amplitudes of HPPH, autofluorescence, PpIX, CpUp and photoproduct of PpIX, respectively. These last three fluorescence components were included because the fluorescence spectra of the patients (especially Patient-2) showed distinct porphyrin peaks at approximately 635 nm and 705 nm (Figs. 2(c) and 2(d)). These peaks correspond to the emission peaks of PpIX in vivo. The measured tissue fluorescence ($F_{data}$) from 600nm to 770nm was fitted to the modeled tissue fluorescence ($F_{model}$) by minimizing $\chi^2 = \sum_{i=1}^{N} ((F_{data}(i) - F_{model}(i)) / \sigma(i))^2$, where $\sigma(i)$ is the standard deviation of each binned pixel. Here, one should note that extracted spectral amplitudes do not correspond to absolute concentrations, since raw fluorescence signal is affected by the optical properties, especially around 410 nm. HPPH fluorescence quantification can be improved by dividing the fitted HPPH amplitude ($C_{HPPH}$) by the autofluorescence amplitude ($C_{Auto}$) under the assumptions that the autofluorescence itself does not bleach and that both the HPPH and the autofluorescence intensities are equally affected by any changes in the optical parameters [34].

2.3 Gel electrophoresis

For determining the cumulative photoreaction in each lesion, biopsies were taken from each lesion after pre- and post-PDT optical measurements for analysis. Briefly, the biopsies were homogenized in Radioimmuno-precipitation buffer and extracted proteins were separated on 6% SDS-polyacrylamide gels. These proteins were then transferred to reinforced nitrocellulose membranes (Optitran, Whatman) and allowed to react overnight with antibodies to STAT3 (Santa Cruz Biotechnology). Enhanced chemiluminescence (ECL) (Pierce
Chemical) was performed to detect immune complexes. ECL images were recorded on X-ray films and pixel values at each band were determined using ImageQuant (Amersham Biosciences). Oxidative cross-linking induced by PDT was expressed as the percentage conversion of monomeric STAT3 into the dimeric complex I. Human hypopharyngeal carcinoma cells (FaDu), treated in vitro with 200 nanomol/ml HPPH and 3 J/cm² of 665 nm light, were used as positive control.

3. Results and discussion

3.1 Pretreatment contrast and changes in blood flow index (BFI)

Figure 3 summarizes the results for blood flow index of the lesion and periphery for each patient. The Patient-1 had ~3.8 × higher blood flow in the lesion compared to Patient-2 (p<0.05). At pretreatment, Patient-1 showed ~4.4 × higher blood flow in the lesion compared to the surrounding periphery (p<0.001) and Patient-2 showed ~5.6 × lower blood flow in the lesion compared to periphery (p<0.002).

PDT induced changes in blood flow index for both patients but the changes in Patient-1 were more significant. Specifically, Patient-1 showed an ~83% decrease (p<0.001) and Patient-2 showed a ~59% decrease in the lesion (p<0.02). In both cases the periphery did not show a significant change (p>0.05).

3.2 Pretreatment contrast and changes in blood volume fraction and blood oxygen saturation

Figure 4 summarizes the results for blood volume fraction (BVf (%) ) and blood oxygen saturation (S_O₂ (%) ) of lesion and periphery for each patient. Patient-1 had 1.8 × higher blood volume fraction in the lesion compared to Patient-2. Patent-1 also had 2.6 × higher blood volume fraction (p>0.05) in the lesion compared to periphery, while Patient-2 had 1.8 × lower blood volume fraction in the lesion compared to periphery (p~0.02). PDT induced a reduction in blood volume faction from 2.5 (mean) to 1.9 (mean); however, this change was not statistically significant (p>0.05). The blood volume fraction in the lesion of Patient-2 did not change much but the periphery blood volume fraction showed an increase.

At pre-treatment, the blood oxygen saturation was ~74% in the lesion of Patient-1 and ~64% in the lesion of Patient-2 (p<0.005). When compared to surrounding periphery tissue, blood oxygen saturation did not show pretreatment contrast for Patient-1, but was 1.2 × lower (p<0.03) in the lesion for Patient-2. Blood oxygen saturation increased at post-PDT for...
Patient-1 but decreased for Patient-2, though these changes were not statistically significant (p>0.05).

3.3 Pretreatment contrast and changes in HPPH concentration

Figure 5 shows the baseline and changes in HPPH photosensitizer concentration for both patients as obtained by diffuse reflectance measurements. Lesion HPPH concentration in Patient-1 (Fig. 5(a)) and Patient-2 (Fig. 5(b)) was higher than periphery. Pre-treatment HPPH
concentration in lesion for Patient-1 was 0.34 µM and for Patient-2 was 0.10 µM, demonstrating a 3.4-fold difference. The differences in HPPH content between the two patients may be due to differences in tissue/lesion type and site as well as the physiological state of the patients such as peripheral blood flow and anesthesia level, etc. We could not compare the HPPH levels in the peripheral blood since the sample for Patient-2 did not allow reliable analysis.

By comparing HPPH concentrations pre- and post-PDT, it is clear that PDT induced significant photobleaching of the photosensitizer. The measured HPPH concentration decreased in both lesions. Patient-1 showed a 52% decrease (p<0.007) in HPPH concentration in the lesion while Patient-2 showed a 39% decrease in HPPH concentration. Periphery values for Patient-1 showed a decrease trend but remained relatively constant for Patient-2 at post-PDT.

3.4 Pretreatment contrast and changes in fluorescence

As Figs. 6(a) and 6(b) indicate, the lesion of Patient-1 had much higher HPPH fluorescence level (1.13 ± 0.28) compared to the lesion of Patient-2 (0.15 ± 0.05). However, both levels were lower than their periphery values. The observed differences in fluorescence and

Fig. 6. Fitted fluorescence amplitudes before and after PDT. HPPH fluorescence for Patient-1 (a) and Patient-2 (b). Fluorescence ratio (HPPH/Autofluorescence (AF)) for Patient-1 (c) and Patient-2 (d). PpIX fluorescence for Patient-1 (e) and Patient-2 (f). Error bars represent standard error, * represents statistical significance (p<0.05)
reflectance measurements may be due to optical property differences between the lesions and peripheries at both excitation and emission wavelengths and differences in penetration depth; fluorescence signal originates from shallower tissue compared to reflectance signal. Normalizing with reflectance data partially corrects at the emission wavelength range [670-750] nm, but absorption and scattering effects at the excitation wavelength of ~410 nm are more pronounced. In this respect we have adapted our instrument recently to assess attenuation at ~410 nm as well as to excite the HPPH fluorescence at ~665 nm for improved quantification of HPPH fluorescence for our ongoing studies. The “apparent” HPPH fluorescence decreased in both lesions after PDT. The periphery of the lesion of Patient-1 showed a large decrease while the lesion of Patient-2 showed a significant decrease in HPPH fluorescence at post-PDT.

Figures 6(c) and 6(d) show the ratio of HPPH and autofluorescence amplitude. At pre-PDT, the “improved” HPPH fluorescence indicates slightly more fluorescence in both lesions than in the corresponding peripheries, though these differences were not statistically significant. At baseline, Patient-1 had 10.7 ± 1.2 and Patient-2 had only 2.0 ± 0.3 as fluorescence ratio level. Both lesions showed statistically significant reductions in ratio fluorescence: The level of Patient-1 decreased 52.7% and that of Patient-2 decreased 75.1%.

We plotted PpIX fluorescence in Figs. 6(e) and 6(f) as the main porphyrin fluorescence component. The lesion of Patient-2 had more PpIX fluorescence than that of Patient-1. The PpIX levels remained stable at post-PDT, any changes were not statistically significant. The other porphyrin fluorescence components (precursors coproporphyrinogen and uroporphyrinogen and photoproduct of PpIX) also did not show significant changes at post-PDT (data not shown).

**Table 1. Pretreatment contrasts between the two patients. All parameters except blood volume fraction (BVf) showed a significant contrast between two patients.**

| Lesion Type | BFI (a.u.) | BVf (%) | StO2 (%) | cHPPH (µM) | HPPH/Auto (a.u.) |
|-------------|------------|---------|----------|------------|-----------------|
| P1 CIS      | 6.7 ± 2.8  | 2.5 ± 0.7| 74 ± 2   | 0.34 ± 0.02| 10.7 ± 1.2      |
| P2 Dysplasia| 1.8 ± 0.5  | 1.3 ± 0.2| 64 ± 3   | 0.10 ± 0.03| 2.0 ± 0.3       |

We summarized the PDT-relevant pre-treatment contrasts (mean ± standard error) in diffuse optical parameters in Table 1. All parameters except BVf were significantly different between the lesions. The lesion of Patient-1 (P1) had more favorable properties related to accumulated local PDT dose, since its photosensitizer content as well as blood flow, blood volume and blood oxygen saturation were higher than Patient-2 (P2).

Pretreatment values as well as PDT induced changes in these parameters can affect accumulated local dose. Table 2 summarizes the changes in the quantified parameters caused by PDT. Changes in photosensitizer (photobleaching), blood flow and blood volume were significantly higher in Patient-1 (P1) than in Patient-2 (P2), but the changes in blood oxygen saturation were similar for both patients, though the trend showed different polarity: P1 showed an increase trend and P2 showed a decrease trend. Changes in blood flow index (BFI)

**Table 2. PDT-induced changes in extracted parameters for Patient-1 (P1) and Patient-2 (P2), BFI and HPPH/Auto were significant for both patients while changes in cHPPH were only significant for Patient-1.**

| Lesion Type | STAT3 (%) | Δ BFI (%) | Δ BVf (%) | Δ StO2 (%) | Δ cHPPH (%) | Δ HPPH/Auto (%) |
|-------------|-----------|-----------|-----------|------------|-------------|-----------------|
| P1 CIS      | 35        | 83.4      | 23.0      | +15.2      | 51.8        | 52.7            |
| P2 Dysplasia| 0.3       | 59.2      | 7.5       | -17.0      | 38.6        | 75.1            |
and the ratio of HPPH to autofluorescence (HPPH/Auto) were significant for both patients while changes in $C_{ppnh}$ were only significant for Patient-1.

Table 2 and Fig. 7 display the STAT3 cross-linking for these two patients. We previously demonstrated that STAT3 cross-linking is a molecular measure of the accumulated local PDT dose and photoreaction [11,12]. As summarized in Table 2, Patient-1 had 35% cross-linking indicating a highly efficient photoreaction and accumulated PDT dose compared to Patient-2 which had only 0.3% cross-linking, demonstrating a ~100 fold difference in photoreaction between the patients.

Our noninvasive, diffuse optics based measurements indicated that blood flow, blood oxygenation, HPPH concentration and HPPH fluorescence contrast at pretreatment, as well as PDT induced changes in these parameters, were significantly different between these lesions. At pre-treatment, blood flow, blood volume fraction and HPPH concentration were higher in the lesion compared to periphery for Patient-1, while the lesion of Patient-2 demonstrated significantly lower blood flow, blood volume and blood oxygen saturation compared to periphery. Both patients showed higher HPPH concentration in the lesion than periphery, but the lesion HPPH concentration of Patient-1 was much higher than in Patient-2. Since local PDT dose is proportional to local light dose and photosensitizer dose, pre-treatment photosensitizer contrast can be a strong indicator of effective PDT [3,5,35]. It should be stressed that blood flow, blood volume and HPPH concentration were quantified by totally different techniques, thus it is interesting to note that high blood flow is related to both high blood volume and HPPH content. Blood volume (and blood flow) was higher in Patient-1 who had carcinoma in situ (CIS), since microvasculature develops as the disease progresses due to angiogenesis and more microvasculature provides more blood volume (and blood flow). Higher blood flow in Patient-1 likely allowed for higher accumulation of HPPH. We can infer from higher blood oxygen saturation in Patient-1 that the lesion was not oxygen-limited yet in the early phase of its progression, and the oxygen supply was higher than oxygen consumption.

In addition to the HPPH peak near 668 nm, fluorescence signals showed additional peaks near 630 nm for both patients with a much higher signal level for Patient-2. The major natural fluorophores in tissue include collagen, reduced nicotinamide adenine dinucleotide, and porphyrins [36,37]. Compared to the others which mainly emit green fluorescence, porphyrins emit unique red fluorescence above 600 nm, and thus spectral analysis is highly suitable in discriminating porphyrin fluorescence. The porphyrins may be produced by the lesion itself, by microbial activity, or they may be products of cancer metabolites [37]. This natural red
fluorescence has been widely observed in human cancer especially in necrotic and ulcerated lesions, and thus might be an indicator of challenge for effective PDT.

In this ongoing Phase-I trial, the light dose was escalated to find the maximum tolerated dose while the administered drug dose was kept constant. Administered light dose was only 0.1-fold (12%) less for Patient-1 compared to Patient-2 (125 vs. 140 J/cm²) but STAT3 cross-linking was ~100-fold (~12,000%) higher. Thus, the main reason for the differences in the local dose is not expected to be due to the administered light dose. Indeed, our noninvasive measurements suggest that Patient-1 had more favorable PDT-related parameters at pre-treatment (Table 1). The local PDT dose was more likely affected by the pretreatment values of the drug concentration (Patient-1 had 3.4-fold higher drug concentration) and physiological parameters such as higher blood flow and oxygenation at pre-PDT. Differences in HPPH concentration between the patients were likely due to delivery and cellular retention. It is possible that oxygen metabolism was different between the patients, as seen in the oxygenation and blood flow values. Our results highlight the benefits of assessing PDT-related parameters at pre-treatment and support the previous findings by others [3,5,38].

It is clear that diffuse optical spectroscopies can improve the pre-PDT tissue assessment by providing hemodynamic parameters and administrated drug (photosensitizer) concentration. The techniques are noninvasive and near-real time, allowing the collection of many measurements at the operating room, and thus have clear advantages compared to single biopsy sampling. However, for widely spread diseases that occur in the oral cavity, spectroscopic point measurements have limitations and imaging is a more desirable option. After image guidance, spectroscopic measurements can be performed to increase tissue specificity and sensitivity as well as to assess PDT-induced changes. Oral lesions are very challenging since they occur at diverse sites (e.g., tongue, gingiva, lip, palate, etc.) with very different optical properties. Each lesion needs to be investigated on a background of surrounding tissue. For this reason, we compared the lesion with respect to periphery. However, there are limitations to this approach wherein there were only three point measurements of the periphery. Compared to well-established solid tumors there is no well-defined “periphery” tissue that can be clearly demarcated by white-light examination. More sampling at the periphery can help reduce this sampling error. Thus, we will be doing more measurements in the periphery in our ongoing studies.

4. Conclusion

In summary, our results indicate that substantial heterogeneity exists in the physiology of oral lesions, affecting drug and oxygen delivery, and that these factors determine the effective PDT dose. We show that these factors can be monitored non-invasively in real time, which ultimately may improve treatment delivery. We acknowledge that sampling of the biopsy tissue and variation of the delivered dose can contribute to the variations in the local dose. It will be interesting to explore the predictive power of these noninvasive indices compared to STAT3 analysis of biopsies. A statistically valid number of patient measurements has potential to assist in this endeavor and will allow better discrimination of patients with respect to local dose as well as PDT induced changes.

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