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Review Article
Implicit and Explicit Dosimetry in Photodynamic Therapy: a New Paradigm

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Abstract. Dosimetry for photodynamic therapy (PDT) is becoming increasingly complex as more factors are identified which may influence the effectiveness of a given treatment. The simple prescription of a PDT treatment in terms of the administered photosensitizer dose, the incident light and the drug-light time interval does not account for patient-to-patient variability in either the photosensitizer uptake, tissue optical properties or tissue oxygenation, nor for the interdependence of the photosensitizer-light-tissue factors. This interdependence is examined and the implications for developing adequate dosimetry for PDT are considered.

The traditional dosimetric approach, measuring each dose factor independently, and termed here 'explicit dosimetry', may be contrasted with the recent trend to use photosensitizer photobleaching as an index of the effective delivered dose, termed here 'implicit dosimetry'. The advantages and limitations of each approach are discussed, and the need to understand the degree to which the photobleaching mechanism is linked, or 'coupled', to the photosensitizing mechanism is analysed.

Finally, the influence of the tissue-response endpoints on the optimal dosimetry methods is considered.

INTRODUCTION

Photodynamic therapy (PDT) of malignant lesions involves administration to the patient of a photosensitizer, a time delay to allow adequate concentration of the drug in the tumour, followed by irradiation of the target tissue volume by light of a wavelength appropriate to activate the photosensitizer efficiently (1–3). The consequent photochemical damage results in tissue necrosis, by direct tumour cell kill and/or by vascular damage leading to ischaemic necrosis (4). For most photosensitizers, it is believed that the PDT effect is mediated by the production of highly active singlet oxygen, $^1O_2$, formed by energy transfer from the excited-state photosensitizer to molecular oxygen in the tissue.

In its simplest terms, a PDT treatment may be described by specifying the administered photosensitizer 'dose' (eg mg kg^{-1} body weight), the incident light 'dose' (eg J cm^{-2}) and the drug-light time interval. Historically, and indeed currently, most clinical protocols still utilize only these three prescribed treatment parameters, despite the fact that there are many additional factors which may influence the effective 'dose' actually delivered to any particular lesion, including:

(1) The subject-to-subject variation in specific tumour uptake of photosensitizer (5–8);
(2) The large range of (wavelength-dependent) optical absorption and scattering coefficients of different tissues, which determine the light penetration and distribution in the target volume (9, 10);
(3) The variability in tissue oxygenation (11), which affects the photodynamic efficiency;

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(4) Changes in light penetration during irradiation, due primarily to rapid PDT-induced blood flow changes (12, 13);
(5) 'Self shielding', which occurs with second-generation photosensitizers of large molar extinction coefficient, and which limits the light penetration due to the added absorption of the photosensitizer itself in the tissue (14);
(6) 'Photobleaching', shown by many photosensitizers during light irradiation (2, 5–8, 15–17), which may reduce the concentration of (photo-active) photosensitizer in the tissue during irradiation; and
(7) Photochemical depletion of oxygen in tissues under irradiation at high light fluence rates, leading to reduced photodynamic effect (18, 19).

Thus, there is a large gap between current clinical dosimetry and a complete and comprehensive description of an actual PDT treatment. While clinical treatments will, no doubt, continue to be improved empirically, this gap is now a major impediment to progress, particularly in understanding how to optimize and standardize treatments with the numerous new photosensitizers which are coming into clinical use (1, 3, 20).

Two main challenges to improving PDT dosimetry can be identified, namely:

(1) To develop methods, and corresponding instrumentation, to measure the various dose factors in individual patients; and
(2) To understand how, both in principle and in practice, these dose-modifying factors fit together to determine the effective delivered dose, to correlate with the tissue reponse.

This paper will examine the second issue. As new dose-modifying factors are revealed, and as the interdependency of these factors becomes more apparent, new paradigms are needed to tackle the increasing complexity of PDT dosimetry. This paper will introduce the concept of 'implicit' vs 'explicit' PDT dosimetry, and discuss the advantages and limitations of each. It is not the objective here to present specific solutions to the many problems in PDT dosimetry, but rather to introduce a new way to view PDT dosimetry in its broadest sense. This requires first an examination of the interdependency of the PDT treatment factors.

INTERDEPENDENCE OF PDT DOSIMETRY FACTORS

Figure 1 illustrates the major interdependencies that have been identified to date between the three fundamental treatment variables; light, photosensitizer and oxygen. As can be seen, for each of these there are various possible measures (metrics) which can be applied. For example, in the case of the photoactivating light, the parameters which may be involved are: the incident fluence and, possibly, fluence rate; the spatial distribution of the light in the target tissue volume; and the temporal characteristics of the light (pulsed vs continuous irradiation). Similarly, for the photosensitizer, in addition to the administered amount of drug (mg kg^{-1} body weight or m^{-2} body surface), the consequent concentration and microdistribution of photosensitizer in the target and adjacent normal tissues are important. For oxygen, the pre-treatment concentration and microdistribution, and repletion rate are relevant, as is the vascular status before and during treatment, since these determine the oxygen available to participate in the photodynamic action. In defining clinical protocols for PDT, it has been usually considered that the light and photosensitizer (and oxygen) are independent treatment variables. However, in reality, each may affect and be affected by (changes in) the others, as in the following cases.

Effect of light on photosensitizer

The photosensitizer can be photobleached, either permanently and/or transiently, by the treatment light. (Note that the term 'photobleaching' is variously used to denote actual photochemical destruction of the photosensitizer or simply decreased optical absorbance and/or fluorescence, which may not be equal and which do not necessarily involve molecular decomposition. In this paper, the term will usually refer to loss of the measurable fluorescence emission of the photosensitizer and it will be assumed that this corresponds to loss of photodynamic activity.) In the case of permanent photobleaching, the concentration of photo-active photosensitizer in the target tissue decreases during the light irradiation. Transient photobleaching is the result of photosensitizer ground-state depletion, and is usually only significant using short

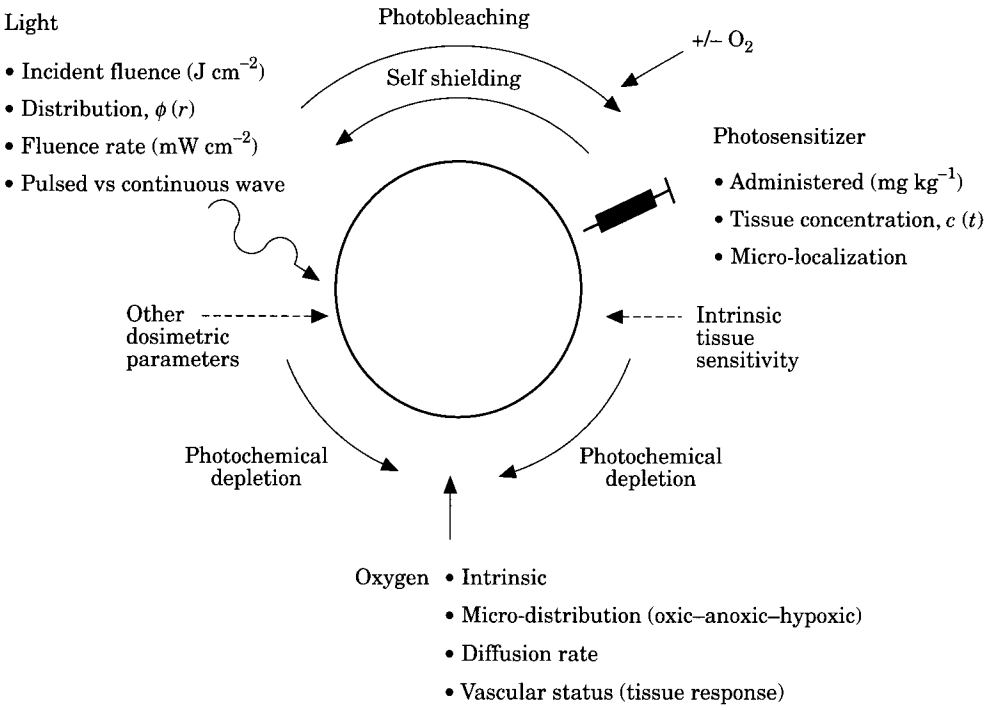


Fig. 1. Diagram illustrating the interdepency of the different dosimetry factors (photosensitizer-, light- and oxygen-related) involved in the photodynamic therapy response of tissue in vivo. The curved arrows indicate the interdependent mechanisms: eg the light fluence affects the photosensitizer through photobleaching; light and photosensitizer together affect the tissue oxygenation through photochemical depletion. As also indicated, the intrinsic photodynamic sensitivity of the tissue (31–34) determines the effectiveness of the resultant combined dosimetric variables and there may be other dosimetric factors (eg tissue temperature) which are not included here.

(sub-microsecond), high peak power pulsed irradiation (21, 22). Since, for any given irradiation technique, there are usually substantial light fluence-rate gradients within the tissue due to the limited penetration of the light, the rate of photobleaching will not be uniform. An example of this is shown in Plate 1(b) where, in the treatment of a gastrointestinal lesion, the photosensitizer, as monitored by its fluorescence endoscopic image, was bleached only in part of the target volume. The corresponding pre- and post-irradiation fluorescence spectra are presented in Fig. 2.

A critical issue for PDT dosimetry using photobleaching as a dose metric is whether or not the photobleaching is oxygen, and/or singlet oxygen, dependent or independent, as discussed below.

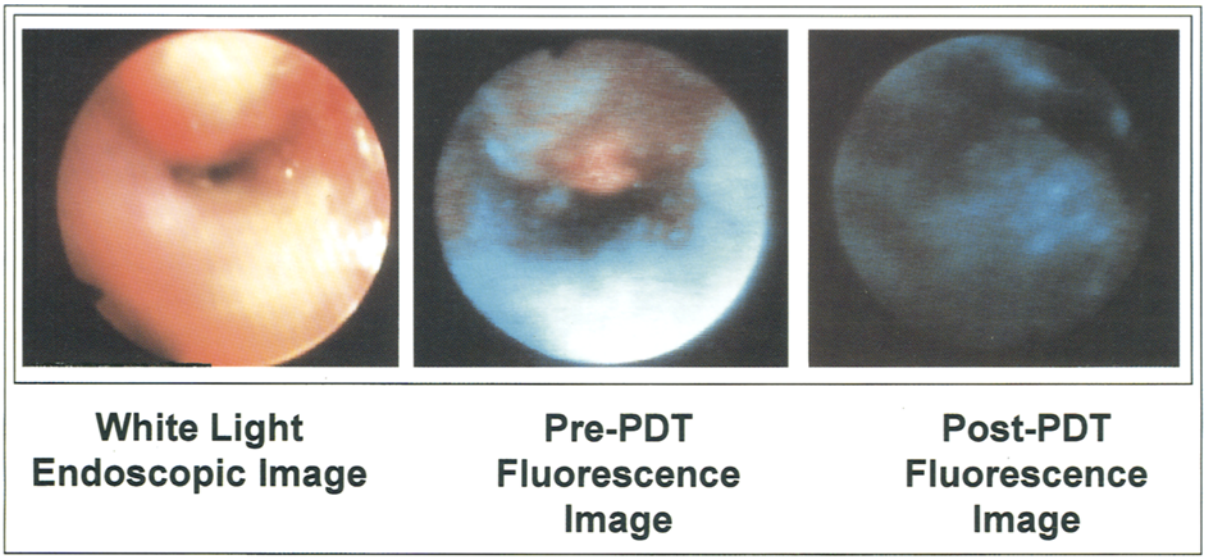
Effect of photosensitizer on light

As was recognized a decade ago (14), with second-generation photosensitizers having a high extinction coefficient at the treatment wavelength, the absorption due to the photosensitizer itself adds to the intrinsic tissue absorption, thereby reducing the penetration of the light ('self-shielding' effect). Thus, the effective treatment volume decreases. This effect may be partially countered by the photosensitizer photobleaching, so that the light and photosensitizer have a complex interplay (which may depend also on the oxygenation) during irradiation, which is not reflected simply in the initial values of each. Other factors may set an upper limit on the useful tissue concentration of photosensitizer, which may then be too low to produce a significant self-shielding effect: for example, where photochemical depletion of oxygen is the limiting factor in the photodynamic action, such that further increase in photosensitizer concentration does not alter the photodynamic effectiveness.

Effect of light+photosensitizer on tissue oxygenation

If the photosensitizer concentration and light fluence rate are high enough, it has been shown (18), both theoretically and experimentally, that photochemical depletion of tissue

(a)



(b)

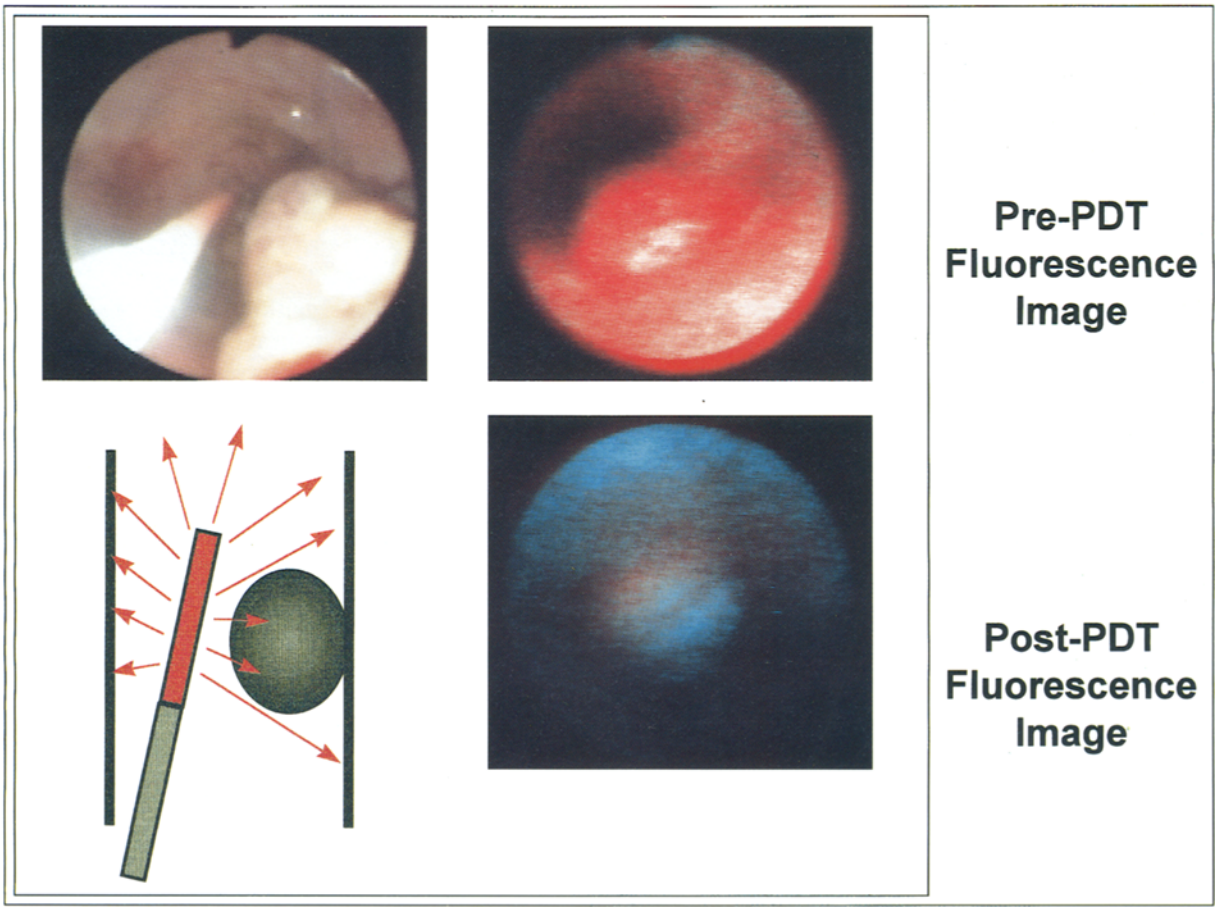


Plate 1. (a) White-light endoscopic image and fluorescence endoscopic images of an oesophageal lesion in a patient before and after Photofrin-photodynamic therapy (PDT) treatment. The photosensitizer appears to be photobleached uniformly throughout the tumour. (b) Similar images, plus a schematic showing the approximate irradiation geometry, in a second oesophageal patient, showing a region at the tumour base with residual Photofrin fluorescence after treatment. Treatment conditions: 2 mg kg^{-1} Photofrin i.v., ~100 J cm^{-1} of 630 nm light at 24 h. Note that the interpretation of such photobleaching images, for example, whether or not the zone with residual fluorescence is 'undertreated', is particularly complicated with Photofrin since this comprises multiple porphyrin components of different pharmacokinetics, photodynamic activities, fluorescence yields and photobleaching rates.

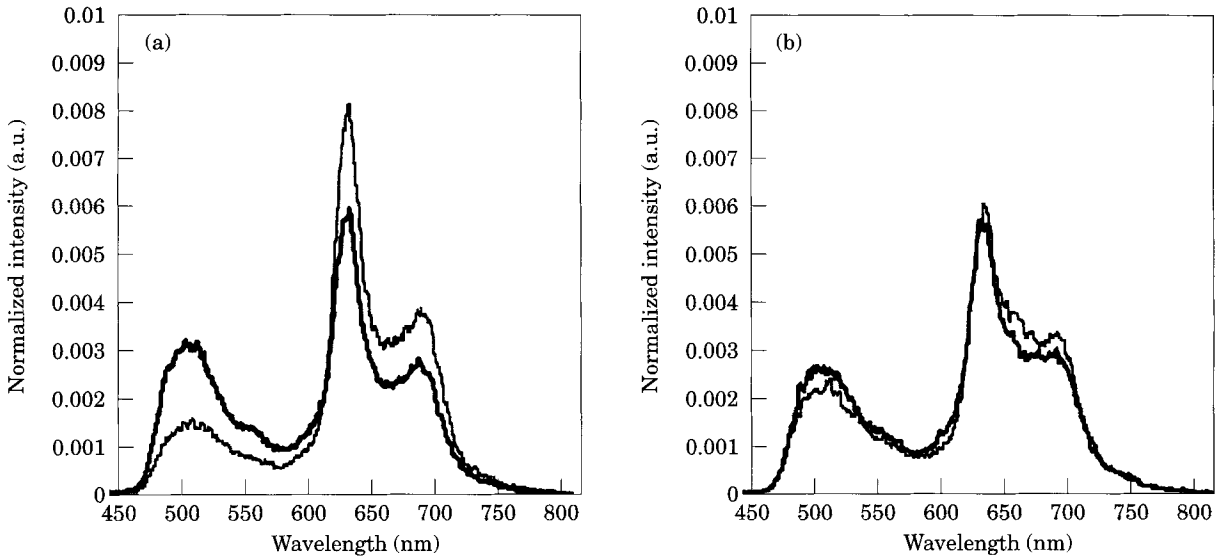


Fig. 2. Corresponding point fluorescence spectra for Case b of Plate 1(a) before and (b) immediately after light irradiation, measured using an optical multichannel analyser with a fibre-optic probe placed on the tumour tissue surface. Note the change in the spectral shape with photobleaching. Each set of spectra has been normalized to the same area under the curve, since the endoscope-tissue distance, and hence the excitation intensity, was not constant between procedures. Thick line, normal; thin line, tumour.

oxygen can occur. For oxygen-dependent photosensitization, this results in a reduced photodynamic effect. The extent to which this occurs depends also on how well perfused the tissue is, and on the oxygen diffusion rate from capillaries. The effect may be mitigated either by reducing the light fluence rate or by an interrupted irradiation regimen of light–dark cycles (typically 2–30 s) to allow re-diffusion of oxygen during the dark phases, in each case maintaining the same total fluence delivered.

The tissue oxygenation can also be altered during treatment if there is an acute vascular response. It is known (4) that the severity of the vascular response (and its contribution to the resultant tissue damage relative to direct tumour cell killing) depends on the tissue and on the photosensitizer parameters: delivery vehicle, route of administration and drug-light time interval.

Effect of tissue blood oxygenation and blood content on light and photosensitizer

As mentioned previously, the photobleaching of the photosensitizer may be oxygen dependent, so that the photobleaching rate can change if the tissue oxygenation alters due either to photochemical depletion or to changing blood flow. An altered tissue blood volume can also affect the light penetration, by increasing or decreasing the absorption due to (oxy)haemoglobin (23, 24).

Thus, there is a multiplicity of ways in which the primary dose parameters affect, and are affected by, each other and by the response of the tissue during the PDT treatment. How then can this interdependency be taken into account in PDT dosimetry? The first option is to measure each parameter directly and independently (including measurements during treatment) and to build a resultant dose metric by combining these, using some model of how the photodynamic response depends on the variables. This will be referred to here as 'explicit dosimetry', since each dose variable is measured explicitly. The second option, 'implicit dosimetry', is to use a metric (eg photobleaching) which implicitly incorporates some, or preferably all, of the dose parameters, while not necessarily measuring any of them directly.

The advantages and disadvantages of each form of dosimetry are summarized in Table 1 and will be discussed below.

EXPLICIT DOSIMETRY

The explicit aproach to PDT dosimetry, which has been most commonly used to date, is illustrated in Figs 3(a) and 4(a) by the dose metrics employed. In its simplest form, this involves

Table 1. Advantages and disadvantages of explicit vs implicit dosimetry

	Explicit	Implicit
+	¶ No strong a priori assumptions ¶ Photobleaching not necessary	¶ Incorporates 'all' photophysical/photochemical/photobiological factors ¶ Technically simple: eg fluorescence measurements only
−	¶ Need model(s) to combine all factors ¶ Technically complex: complete data set may not be possible ¶ Does not incorporate/account for microdosimetric factors (eg local O_2 depletion)	¶ Assumes common pathway(s) for photobiological and/or photosensitizer fluorescence changes ¶ May need additional information to make quantitative (eg tissue optics) ¶ Not clear how to define effective 'dose'

measuring the average photosensitizer concentration in or around the target tissue just prior to light treatment, and the light fluence (rate) at some points within or around the target volume. This has the merit, compared with simply using the prescribed photosensitizer and delivered light doses, that it takes into account the patient-to-patient variability in photosensitizer pharmacokinetics and tissue optical properties. Substantial progress has been made in developing techniques and instruments to make such measurements either invasively or non-invasively (25–28). The main a priori assumption involved is that the product of local photosensitizer concentration and light fluence is a predictor of tissue response. Certainly, the present authors (31, 32) and others (15, 33, 34) have shown in a number of studies of PDT threshold dose, which is calculated from explicit measures of the local light fluence and photosensitizer concentration, that this can be accurate for specific tissue-photosensitizer combinations, using gross volume necrosis as a response endpoint. There are, however, reports, both in vivo (35), and in vitro (36), where the drug-light product was not predictive of outcome (excluding 'trivial' cases such as reciprocity failure due to photobleaching not being taken into account: see below). In particular, the effect of photochemical depletion of local oxygen is not accounted for by monitoring only the photosensitizer and light fluence. It is possible to add dynamic tissue oxygenation (pO_2) measurements, although at present this involves invasive single micro-electrodes or complex and expensive clinical instruments, such as the Eppendorf probe (29), and measurements can only be made at a limited number of positions. A promising, non-invasive optical method based on oxygen-dependent quenching of the phosphorescence of an exogenous probe molecule (eg metal porphine derivatives) has been reported recently (30), which may make oxygen mapping more practical.

An advantage of the explicit approach is that it can be used even if there is no trackable photosensitizer change, such as photobleaching, as is required for implicit dosimetry. Explicit dosimetry can be refined to take into account factors such as photosensitizer photobleaching or changing optical properties during treatment (21, 37). However, the approach can be technically complex, especially if the objective is to obtain a complete set of dose factor measurements. It also requires a model for which all the dose factors can be used as input, which presently exists only for some relatively crude biological endpoints. More fundamentally, however, a major limitation is that the explicit approach does not take into account microdosimetric factors or the 'downstream' photophysical/photochemical processes in the photodynamic pathway. These important elements are built into the implicit dosimetry concept, at least in principle.

IMPLICIT DOSIMETRY

The concept of implicit dosimetry is to use a metric which, as far as possible, incorporates all of the response-determining treatment factors and their interdependencies, thereby avoiding the need to measure these factors separately and for a model in which to combine them. In essence, as illustrated in Figs 3(b) and 4(b), the objective is to move the dose

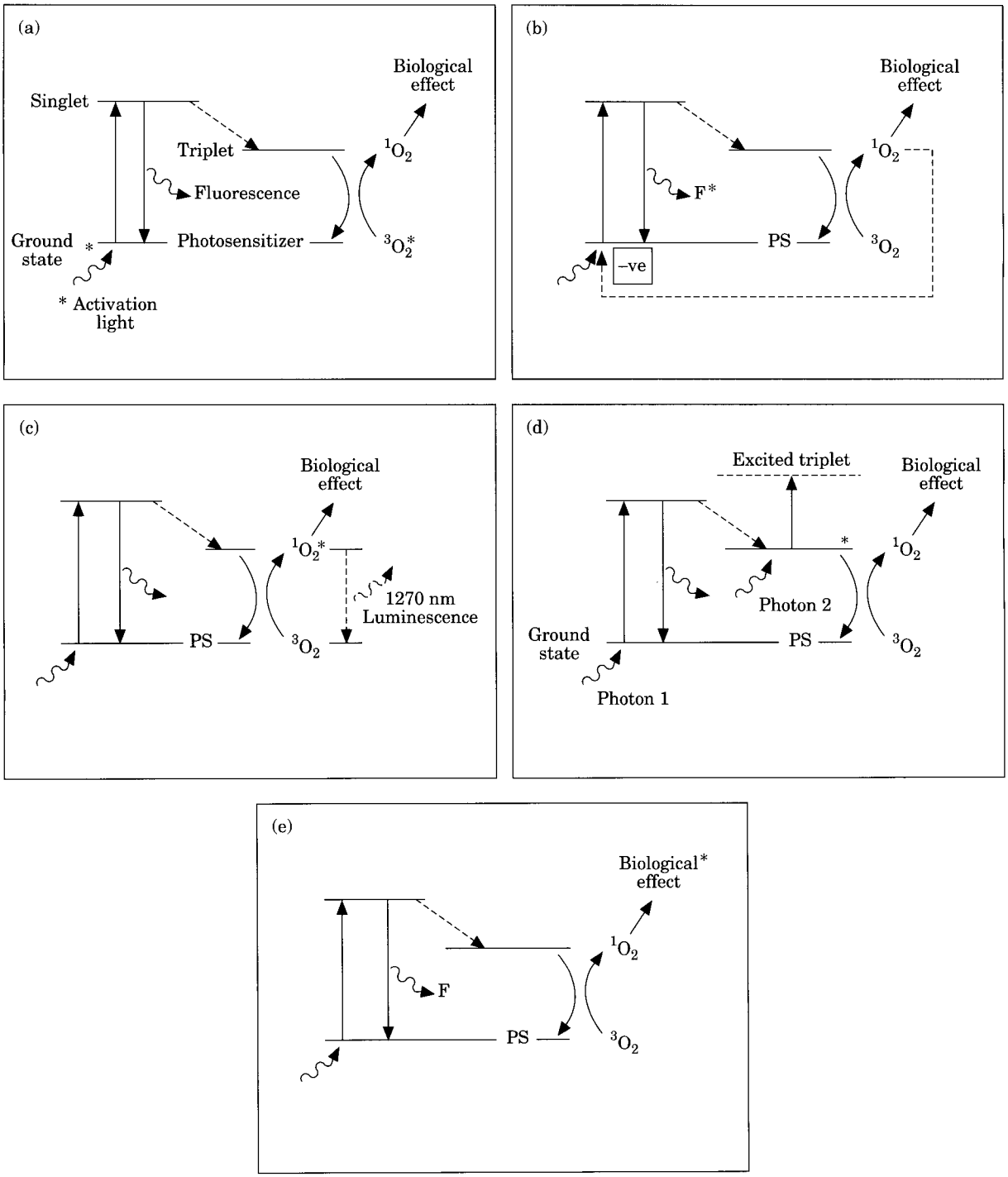


Fig. 3. Energy-level diagrams, indicating (*) the dose metrics used in different dosimetric approaches ($^1O_2$-mediated pathways). (a) Explicit metrics: light fluence (rate), photosensitizer concentration, tissue oxygenation. (b) Implicit metric: photosensitizer photobleaching (rate). (c) Direct metric: singlet oxygen concentration, eg by 1270 nm luminescence. (d) Triplet-state metrics: photosensitizer triple-state concentration, generation rate. (e) Photobiological metrics: measures of biochemical/biological changes during or immediately after irradiation.

metric further along the photophysical/photochemical/photobiological pathway.

Implicit dosimetry depends on the use of photosensitizer photobleaching to generate the dose metric. This is most simply carried out in vivo using fluorescence measurements, because of the high sensitivity of this technique even for relatively low photosensitizer concentration or fluorescence quantum yield. It is also possible to use in vivo absorption spectroscopy (determined indirectly via, for example, diffuse reflectance spectroscopy) to monitor photodestruction of the photosensitizer (28, 38). This is generally less sensitive

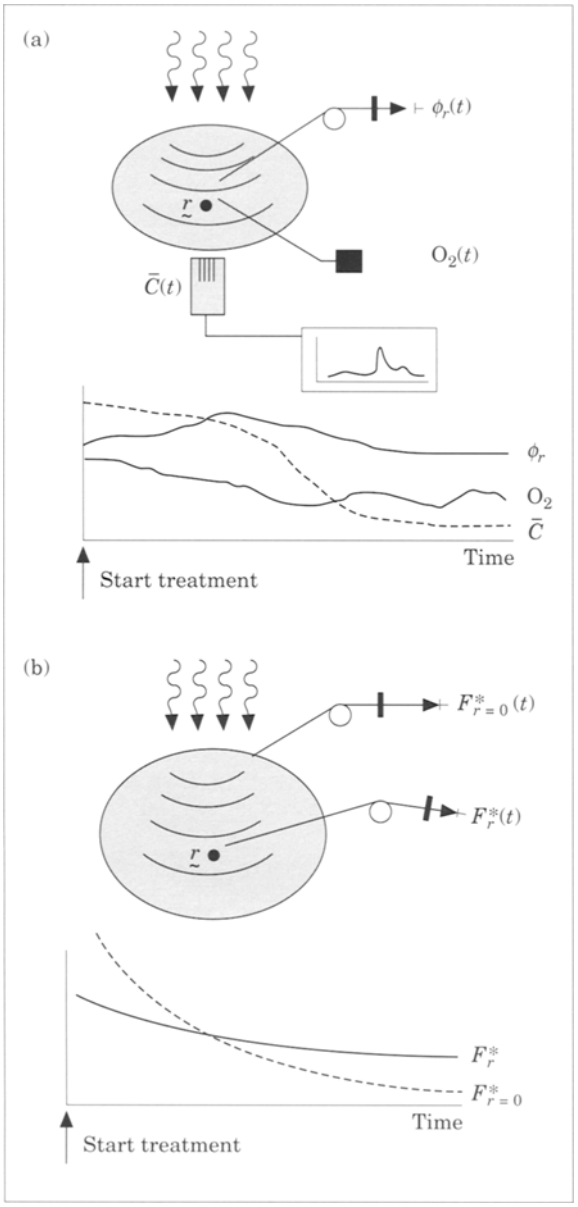


Fig. 4. Schematic illustrations of (a) explicit and (b) implicit dose measurements. Measurements are shown made either at the target tissue surface or at a point of interest, *r*, within the tissue volume. φ is the light fluence, *C* is the (tissue average) photosensitizer concentration and *F** is the apparent, measured photosensitizer fluorescence signal.

than fluorescence measurements, but may give complementary information: for example, if photobleaching leads to loss of fluorescence but not to proportional loss of photodynamic activity (39). Here, for simplicity, only implicit dosimetry via fluorescence monitoring will be discussed.

The principle is to use some measure, F^*, of the photosensitizer fluorescence in vivo (normalized to the excitation light irradiance or power) and to monitor the decrease in F^* during irradiation. The extent to which the photosensitizer is photobleached is related to the photochemical activation of the drug and thereby, it is assumed, to the photobiological effect on the tissue. However, two distinct cases must be considered, depending on whether or not the photobleaching is caused, in whole or in part, by the $^1O_2$ (or, more generally, by the cytotoxic photoproduct) which is responsible for the photodynamic effect. The terms 'coupled' and 'uncoupled' will be used here to indicate this. Note, however, that the fraction of the $^1O_2$ molecules which interact with the photosensitizer and result in photobleaching is probably $\ll 1$ for most photosensitizers, independent of the degree of coupling, which represents the fraction of photobleaching interactions due to $^1O_2$ compared to those due to other processes not involved in the photodynamic effect. Coupled photobleaching is indicated in Fig. 3(b) by the dashed line, representing a negative feedback from the $^1O_2$ production to the photosensitizer ground state.

The degree of coupling makes a significant difference to how photobleaching measurements should be interpreted and applied as a PDT dose metric. Two extreme cases serve to illustrate this point. If the photodestruction is completely uncoupled, then the photobleaching simply results in loss of photo-active drug during the irradiation. Consequently, an appropriate metric is the area under the fluorescence photobleaching curve and, as illustrated in Fig. 5, the effective PDT dose is then some multiplicative function of the incident light fluence, φ_o, the integrated fluorescence, $\int F^*(\varphi)d\varphi$, and the tissue oxygenation, with the dose normally increasing with increase in each of these factors. (In the case of constant treatment light irradiance or power, $\int F^*(\varphi)d\varphi = \int F^*(t)dt$, where t is the light exposure time. However, the more general form, integrating over fluence, will be used here.) In this case, strong photobleaching reduces the photodynamic effect (compared to the case of no photobleaching), because of loss of photo-active sensitizer. Conversely, if there is full coupling (100% feedback), the greater the singlet oxygen production (and resulting photodynamic effect), the faster the photobleaching. In this case a high degree of photobleaching indicates a strong photodynamic effect. It is likely in practice that there will be always some degree of coupling since reactions involving, for example, singlet oxygen may affect a wide range of chemical structures.

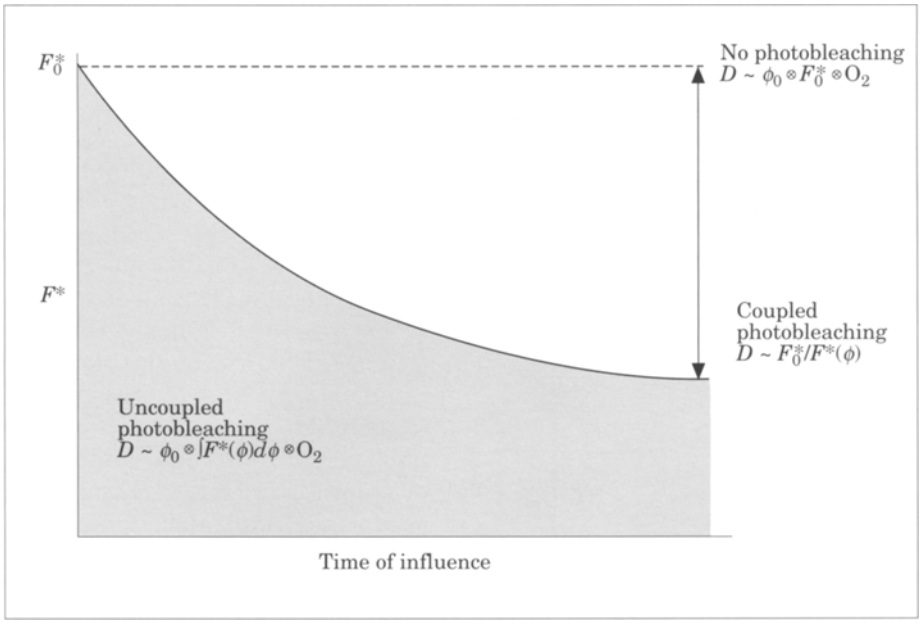


Fig. 5. Implicit dosimetry metrics defined with reference to the measured fluorescence photobleaching curve F^* (t or φ). For fully uncoupled photobleaching, the area under the fluorescence curve, $\int F^*(\varphi)d\varphi$, is a measure of the total effective photosensitizer concentration, so that the metric is a multiplicative function (denoted by $\otimes$) of this quantity, the integrated light fluence (approximately proportional to φ_o) and, if measured, the tissue oxygenation. For fully coupled photobleaching, one possible metric is the ratio of the fluorescence signal at the end of treatment to the initial value, as indicated. In cases where the photosensitizer does not photobleach, implicit dosimetry does not apply, and the dose metric is given by the product of the initial photosensitizer fluorescence, F^*_o ($\propto$ initial photosensitizer concentration) and the total light fluence.

It is important to note that, regardless of whether it is coupled or uncoupled, the photobleaching can be incorporated into a dose metric which is predictive of the tissue response. It is then the *form* of the dose metric with which this response correlates that distinguishes between the two cases.

Figure 5 illustrates the situation at a given light fluence rate. However, if, for example, the light fluence rate is reduced under conditions where there is significant photochemical oxygen depletion at high fluence rate, the singlet oxygen generation rate increases, resulting in an enhanced photodynamic effect. If the photosensitizer is fully uncoupled, its measured photobleaching rate will simply decrease proportionally with the fluence rate. However, with a coupled photosensitizer, the photobleaching rate may increase, due to the higher instantaneous $^1O_2$ concentration.

As an example of how an implicit dose metric can be derived, consider the chemical kinetics for the concentration of sensitizer ground state, $[S_o]$, and biological targets, $[A]$. Using the notation of Georgakoudi et al (40):

$$\frac{d[S_o]}{dt} = -k_{os}[S_o][^1O_2] \tag{1}$$

and:

$$\frac{d[A]}{dt} = -k_{oa}[A][^1O_2] \tag{2}$$

where $[^1O_2]$ represents the instantaneous singlet oxygen concentration at time t, and k_{os} and k_{oa} are the rate constants for interaction of the singlet oxygen with the sensitizer and the biological targets, respectively. It can then be shown that:

$$\frac{[A](t)}{[A](0)} = \left\{ \frac{[S_o](t)}{[S_o](0)} \right\}^{k_{oa}/k_{os}} \tag{3}$$

Assuming that the observed fluorescence signal, $F^*(t)$, is proportional to $[S_o](t)$, this becomes:

$$\frac{[A](t)}{[A](0)} = \left\{ \frac{F^*(t)}{F^*(0)} \right\}^{k_{oa}/k_{os}} \tag{4}$$

Thus, if the fraction of 'surviving' targets is a good predictor of eventual biological response, the ratio of the measured fluorescence, after a given treatment time or corresponding light fluence, to its initial value would be an appropriate dose metric for coupled photobleaching. Note that, under these several simplifying assumptions, it is not necessary to measure the

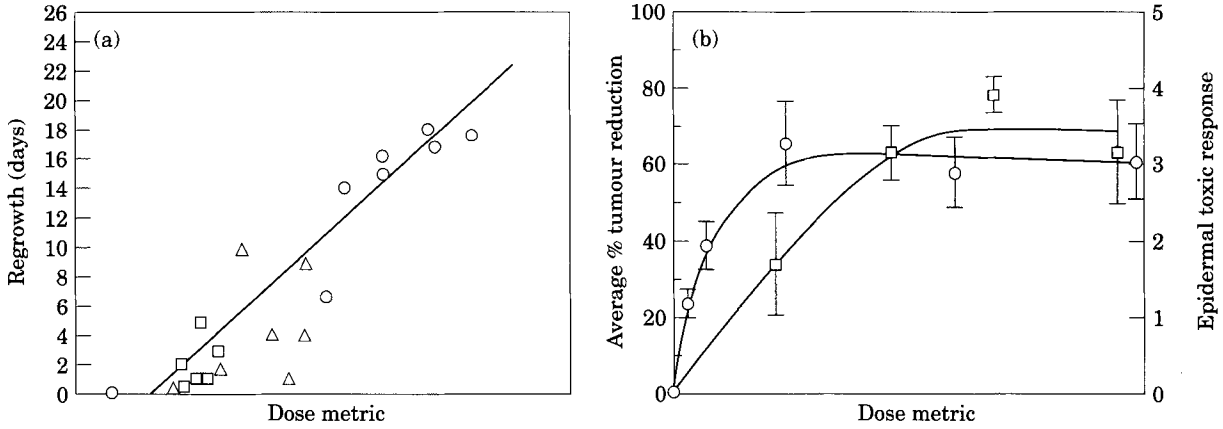


Fig. 6. Examples of in vivo photosensitizer fluorescence measurements correlated with the photodynamic therapy (PDT) tissue response. (a) Tumour response vs the dose metric $[\varphi_0 \cdot \int F^*(t)dt]$ for Photofrin-mediated PDT in a transplanted rodent tumour model, with the photosensitizer fluorescence (630 nm excitation, 690 nm detection) measured at the tumour base. Various combinations of injected photosensitizer dose and incident light fluence were used, as indicated (unpubl. data, courtesy of Dr D. Doiron, PDT Inc., CA, USA and Dr C. Gomer, Childrens Hospital of Los Angeles, CA, USA). △, 2.5 mg kg^{-1}, 300 J cm^{-2}; □, 6.0 mg kg^{-1}, 300 J cm^{-2}; ○, 7.5 mg kg^{-1}, 100 J cm^{-2}. (b) Tumour and normal skin response vs the dose metric $[\varphi_0 \cdot F^*(t=0)]$ for topical ALA-mediated PDT in human T-cell lymphoma patients, with the photosensitizer fluorescence measured at the tissue surface (unpubl. data, courtesy of Dr A. Oseroff, Roswell Park Cancer Institute, Buffalo, NY, USA). □, % tumour reduction; ○, epidermal toxic response.

light fluence, oxygen concentration or absolute photosensitizer concentration explicitly, and that a measure of the fractional loss of fluorescence signal alone may be used as a predictor of tissue response. It would still be required, however, to establish empirically what fractional loss of fluorescence corresponds to the desired biological endpoint for a given tissue and photosensitizer, so that it remains to be seen how applicable in clinical practice this simple form of implicit dosimetry metric will be.

The fundamental advantage of the implicit dosimetry approach is that it folds together the multiple photophysical/photochemical/photobiological factors involved in the PDT effect. In the case of a fully coupled photosensitizer, a measurement of the relative change in F^* may provide an adquate dose metric, as above. For partially coupled or uncoupled photobleaching, it is also necessary to measure some of the other explicit dose factors, such as the initial photosensitizer concentration or the delivered light fluence. Furthermore, although monitoring F^* is technically simple in principle, it is challenging to make in vivo fluorescence measurements truly quantitative in practice (24, 41, 42).

Recently, as summarized below, there have been a number of preliminary reports using in vivo photosensitizer fluorescence or photobleaching thereof. Although these have not been discussed in terms of implicit dosimetry and have incorporated the photobleaching measurements into different metrics, they both illustrate the potential of the approach and raise some fundamental questions.

EXAMPLES OF IN VIVO PHOTOBLEACHING STUDIES

Surface or interstitial fluorescence monitoring of relative photosensitizer uptake and/or photobleaching during PDT has been reported (6, 7, 16, 43–47), both in pre-clinical animal models and in a limited number of patient studies. Few studies have looked specifically at the correlation between these fluorescence measurements and the resulting photodynamic response of the tissue (38, 49, 50). Figure 6(a) shows an example for a transplanted animal tumour model using Photofrin, where the tumour response was measured following PDT treatment and correlated with in situ fluorescence measurements made with a small isotropic optic-fibre probe at the tumour base during treatment. In this case, a strong correlation was found between the tumour response and the product of the incident fluence and the integrated fluorescence, ie with the dose metric $D \sim [\varphi_0 \cdot \int F^*(\varphi)d\varphi]$. In the context of the implicit dosimetry model, these data are consistent with the Photofrin bleaching being (predominantly) uncoupled in this tissue. However, to reach a more definitive conclusion would require that the treatments be done such that a range of

fractional photobleaching values was achieved between the individual tumours. It was also observed in these experiments (D. Doiron, pers. comm.) that the response did not correlate well with the total light fluence alone, confirming the value of the photobleaching measurements.

Analogous observations have been made by A. Oseroff et al in the response of both normal skin and cutaneous T-cell lymphoma lesions to ALA-mediated PDT, while measuring the initial fluorescence at the tissue surface. This is illustrated in Fig. 6(b), and the data show excellent correlation between the tissue response and the photobleaching dose metric in this patient group. The ALA-induced PpIX was almost fully bleached in these lesions, so that the metric used, $D \sim [\varphi_0 \cdot F^*(0)]$, is proportional to $[\varphi_0 \cdot \int F^*(\varphi) d\varphi]$, again consistent with predominantly uncoupled photobleaching.

Recently, Georgakoudi et al (40) have reported measurements of oxygen concentration at the surface of tumour cell spheroids receiving PDT. The oxygen concentration showed an initial rapid decrease due to photochemical depletion, followed by a slow recovery as the rate of oxygen consumption dropped due to photosensitizer bleaching. With Photofrin, the time dependence of the oxygen concentration was consistent with $^1O_2$-mediated photobleaching, but not with bleaching which depended only on the delivered light fluence, suggesting in this case that Photofrin is a coupled photosensitizer, for which implicit dosimetry should work well. Similar findings were made with the porphyrin photosensitizer PpIX, synthesized from ALA (49).

In the same paper (49), it was demonstrated that earlier measurements of Photofrin photobleaching, such as those of Moan (48), which showed deviation from first-order kinetics, could be fitted assuming $^1O_2$-mediated bleaching. In fact, Forrer et al have suggested that, in the presence of abundant oxygen, the photobleaching is governed by the following equation:

$$\frac{d[S_o]}{dt} = -\frac{S_\Delta \phi_t H \sigma k_{os} [S_o]^2}{k_d + k_{oa}[A]} \quad (5)$$

where S_Δ is the fraction of triplet-state quenching collisions between ground-state oxygen and triplet-state sensitizer resulting in singlet oxygen formation, φ_t is the sensitizer triplet yield, H is the local fluence rate, σ is the

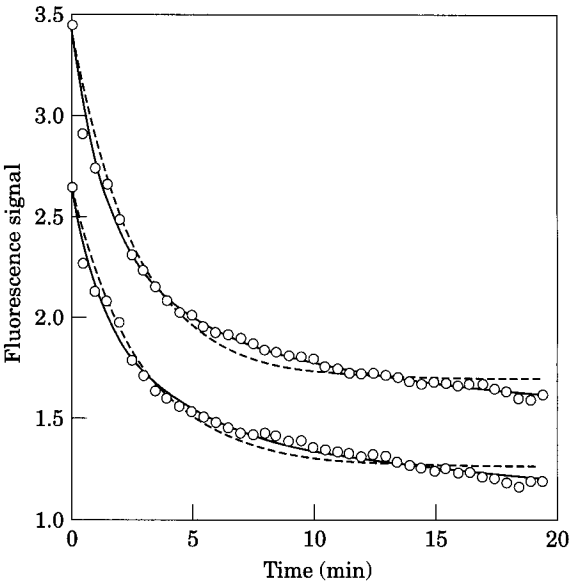


Fig. 7. In vivo F^* measurements made in a Radiation Induced Fibrosarcoma (RIF) mouse tumour model. Measurements were made at 24 h post i.p. injection of 10 mg kg^{-1} Photofrin, during surface irradiation at 630 nm and 100 mW cm^{-2}. The fluorescence at 690 nm was detected using 400 μm diameter optical fibre probes inserted at two different depths in the tumour (upper curves 2 mm, lower curves 5 mm). The dashed and solid lines represent the best fits to the experimental data for first-order and second-order photosensitizer fluorescence decay kinetics, respectively, plus a constant autofluorescence component.

sensitizer ground-state absorption cross-section and k_d is the rate of monomolecular decay of singlet oxygen. If $[A]$ remains approximately constant during treatment, the solution of this equation is:

$$[S_o](t) = [S_o](0)\left[1 + \frac{[S_o](0) S_\Delta \phi_t H \sigma k_{os}}{k_d + k_{oa}[A]} t\right]^{-1} \quad (6)$$

This would also describe the observed fluorescence decay if $F^*(t)$ is proportional to $[S_o](t)$. Forrer et al (49) showed that Equation 6 could be used to describe the measured bleaching of the sensitizer mTHPC in patients receiving PDT of oesophageal lesions. Analogously, Fig. 7 shows the fluorescence measured at 690 nm in a transplanted murine tumour sensitized with Photofrin and irradiated at 630 nm. Data were collected with two optical fibres implanted at different locations in the tumour. As in Forrer et al (49), the observed bleaching kinetics were consistent with Equation 6, but could not be fitted well using first-order kinetics.

Despite these encouraging results, to the authors' knowledge there have been no

published studies to date either to test the implicit vs explicit dosimetry approaches systematically or, with the exception of the recent work in spheroids by Georgakoudi & Foster (51), to assess the degree of coupling/uncoupling of clinical or preclinical photosensitizers. In addition, there are some apparently conflicting data. For example, for ALA-induced PpIX, the observation of second-order oxygen-dependent photobleaching noted above (49) is not in agreement with the authors' interpretation of initial human studies by Oseroff [Fig. 6(b)]. As a second example, Potter et al (pers. comm.) have studied photobleaching, using point fibre-optic surface fluorimetry, of Photofrin in vivo in tumour-bearing mice, both while alive and 20 min following nitrogen asphyxiation. The measured photobleaching rate increased in the latter case. However, as discussed above, Georgakoudi et al (40), using an in vitro tumour cell spheroid model, showed that there was a strong oxygen dependence in the opposite direction in the measured loss of Photofrin phototoxicity during irradiation. The in vivo results may indicate a change in the balance between oxygen-dependent and -independent photobleaching mechanisms. They could also have a component due to changing tissue albedo from the altered blood absorption, which would affect the apparent photobleaching rate at the tissue surface without necessarily a corresponding change in the true photobleaching rate (44). However, unless this latter measurement artefact is the full explanation, it is not clear how to reconcile the in vivo and in vitro observations. These apparent discrepancies point out the need for: (a) studies which are designed explicitly and rigorously to test the implicit dosimetry formalisms; and (b) care to be taken in the methods and reporting of pre-clinical and clinical correlations between photosensitizer fluorescence/photobleaching and tissue PDT response.

In further photobleaching studies in multicell spheroids, Georgakoudi & Foster (51) have also shown that, unlike porphyrin sensitizers (represented by protoporphyrin IX), a Nile Blue Selenium compound was rapidly photobleached via non-oxygen-mediated mechanisms, whereas a Nile Blue Sulphur analogue had quite different oxygen consumption kinetics. Thus, the degree to which oxygen-mediated pathways are involved in the photobleaching and hence, by implication, the degree of photosensitizer coupling involved, is strongly dependent on the photosensitizer class and specific molecular structure.

ISSUES IN THE USE OF PHOTOBLEACHING AS A DOSE METRIC

Although the use of photosensitizer photobleaching in implicit dosimetry is attractive in principle, there are a number of issues which must be resolved for it to become routinely useful.

(1) It is only relevant for photosensitizers which demonstrate significant photobleaching at clinical light fluences. An alternative, if this does not hold, might be to use a second 'reporter' fluorophore which undergoes photobleaching at these fluences.

(2) As mentioned above, it is necessary to know the degree of photosensitizer coupling in the specific tissue environment, which may depend on biophysical factors such as the photosensitizer binding to the tissue substrate and, hence, on the photosensitizer structure, the tissue type and the time interval between photosensitizer administration and light irradiation. For example, the local oxygen concentration may be much higher in lipid (membrane) cellular compartments than in cytosol, which could alter the (coupled) photobleaching rate.

(3) It is not clear exactly how to calculate the effective PDT dose from the photobleaching measurements, particularly if there is partial coupling of the photobleaching. This requires further modelling of the inter-relationships between photobleaching, singlet oxygen generation and biological damage.

(4) The relationship between the true photobleaching rate in solu and the 'apparent' photobleaching rate (for example, measured by a fluorescence probe placed on the irradiated tissue surface or at depth within the tissue such as at the tumour base) depends in a complex way on the tissue optical properties at the fluorescence excitation and emission wavelengths, λ_{ex} and λ_{em}, respectively (24, 41, 44, 49). For example, measuring the fluorescence of ALA-induced PpIX in a rat tumour model, Jacques et al (44) found the apparent photobleaching rate at the tumour surface (630 nm excitation) to be a factor of 5 greater than the true value in solution. Indeed, even placing an invasive fibre-optic fluorescence probe at the point of interest within

the target tissue, as illustrated in Fig. 7, does not completely solve the problem, since the fluorescence signal is still averaged over some tissue- and wavelength-dependent volume surrounding the fibre tip. Thus, it may be necessary also to measure the tissue optical properties at λ_{ex} and λ_{em} in order to correct the measured photobleaching values. This issue is further complicated (a) by the fact that the absorption, fluorescence and/or photodynamic action spectrum of the photosensitizer may change, and in particular the spectral peak(s) may shift, in vivo compared to in solution (52, 53); and (b) by possible treatment-induced changes in the tissue optical properties at λ_{ex} and/or λ_{em}, so that, even with implicit dosimetry, direct monitoring of the tissue optical properties during treatment may be required.

(5) As with any quantitative application of in vivo photosensitizer measurements, the tissue autofluorescence background must be subtracted in order to obtain the signal due to the photosensitizer only. The tissue autofluorescence can be substantial compared to the photosensitizer fluorescence, as seen in Fig. 2(b). This may require making measurements at more than one fluorescence excitation or emission wavelength (15, 42), which complicates the method and instrumentation needed.

(6) Forrer et al (49) have also noted a significant autofluorescence signal decrease using 514 nm irradiation in the oesophagus of patients undergoing PDT treatment with the photosensitizer mTHPC. The decrease was more rapid than the apparent photobleaching of the photosensitizer itself. If this was indeed due to photobleaching of the endogenous tissue fluorophores (as opposed, say, to increased light attenuation at this short wavelength resulting from increased tissue blood content), then it would be critical to monitor the autofluorescence throughout the treatment in order to subtract the autofluorescence contribution from the total fluorescence signal. Partial photobleaching of tissue autofluorescence has also been reported by van der Veen et al (54).

(7) As illustrated also in Plate 1, the photosensitizer components may not photobleach uniformly and the resulting post-photobleaching spectral shape may be different from that pre-treatment. This may be due, for example, to differential photobleaching of separate photosensitizer components, each of which may also have different photodynamic effectiveness, as shown recently in vitro by Bezdatnaya et al (39).

(8) Fluorescent photoproducts may be generated (55–57) which may have different photodynamic effectiveness as well as altering the overall fluorescence spectrum. With respect to both this point and the previous one, Andersson-Engels et al (47) have suggested that monitoring such changes in the shape of the photosen-sitizer fluorescence spectrum could be used for PDT dosimetry.

(9) The relationship between tissue response, for example depth of necrosis, and photosensitizer photobleaching is different for continuous vs pulsed irradiation (21, 22). For the latter, care is then needed in making and interpreting the in vivo measurements so as to take into account the effects of transient photobleaching due to photosensitizer ground-state depletion, which is distinct from the permanent photobleaching. The transient bleaching does, however, depends on the triplet-state lifetime, since this determines the ground-state repopulation rate, and so is indirectly affected by the later stages in the photophysical pathway. Measuring the transient photobleaching may, therefore, provide additional photophysical information.

(10) As pointed out by Stringer et al (16), fluorescence monitoring as a PDT dose metric assumes that the ratio of the quantum yield of fluorescence and triplet-state formation remains constant during phototransformation of the photosensitizer, but this has not been specifically demonstrated to date in vivo. This would influence the use of photobleaching as a measure of cytotoxic photoproduction.

(11) A choice must be made between using the PDT treatment wavelength or some other wavelength for the fluorescence excitation. Since the fluorescence quantum yield is independent of the excitation wavelength, the photobleaching kinetics should not be affected. However, the fluorescence signal strength is different in the two cases, as is the influence of optical attenuation by the tissue. Using the treatment wavelength has the advantages that the measurements can be done without interrupting the treatment and that the tissue 'sampling volume' is comparable to the PDT treatment volume. However, many photosensitizers have relatively small Stokes shifts when activated at long wavelength (as normally used for treatment), which makes separation of the fluorescence emission signal difficult. In addition, the use

of a short wavelength restricts the fluorescence excitation volume, and so provides superior localization for the photobleaching measurements.

ALTERNATIVE DOSE METRICS

There are potential alternatives to the explicit and implicit forms of PDT dosimetry. Those illustrated in Fig. 3(c–e) refer to photosensitization via singlet oxygen as the cytotoxic photoproduct, and only this case will be considered here. The most straightforward dose metric, at least in principle, is to measure directly the putative cytotoxic agent, $^1O_2$, as in Fig. 3(c). This can be done by monitoring the luminescence emission at 1270 nm as $^1O_2$ returns to the triplet ground state. However, while this has been demonstrated in solution, it has not proved possible with current technology to measure the luminescence in cells or tissues in vivo due to the very short (sub-μs) lifetime of $^1O_2$ in biological environments (58, 59), which results from its high chemical reactivity. The development of near-infra-red photodetectors of high sensitivity/low noise may solve this problem in the future. An alternative, indirect method, is to use a 'reporter molecule', eg a fluorophore (60, 61) which is altered by interaction with singlet oxygen.

Another option [Fig. 3(d)] is to measure the photosensitizer triplet-state concentration and lifetime using, for example, transient triplet-state absorption (diffuse reflectance) spectroscopy (62). Following the initial photon absorption of a short light pulse by the photosensitizer ground state, a second pulse is used with a wavelength matched to the triplet-state absorption spectrum. Then, by varying, for example, the time delay between the pulses, the concentration and lifetime of the photosensitizer triplet state in vivo can be monitored, thereby probing indirectly the conversion of molecular to singlet oxygen. To date, few studies of this two-photon/two-colour method have been published, and there are a number of limitations: at present, the pulsed laser technologies are expensive and not suitable for routine clinical use; in order to distinguish the ground-state and triplet-state absorptions, the absorption spectra must have minimal spectral overlap, so that the method cannot be used for all photosensitizers; and the sensitivity may be inadequate for some photosensitizers, since a significant fraction of these molecules must be excited during each light pulse in order to populate the triplet state adequately. However, this approach does provide information further along the photo-activation pathway and, therefore, can yield dose metrics which should have strong correlation with the tissue response. [It is of interest that absorption from the triplet state could itself be expoited to access other photochemical pathways, including the possibility of achieving oxygen-independent photobiological mechanisms (63).]

The last scheme, shown in Fig. 3(e), is the concept of true photobiological dosimetry, ie the use of measurable change(s) in the tissue, leading to, and hence correlated with, the ultimate clinical tissue response. As in, for example, the use of radiological image monitoring of thermal tissue destruction (64), the tissue changes should be detected during or shortly after treatment in order to be maximally useful. The technique required may be tissue dependent: for example, the authors are currently investigating the use of contrast-enhanced computerized tomographic imaging to measure the changes in blood-brain barrier permeability and blood volume during and after PDT in normal brain tissue (65, 66). Other forms of quantitative radiological imaging, such as high-resolution ultrasound or contrast-enhanced magnetic resonance imaging (67), might be used to determine the depth of the immediate PDT effect, which could subsequently be correlated with the tissue necrosis. Similarly, radionuclide imaging may be used to monitor changes in blood perfusion (68), which can correlate with tissue response, although the applicability of this will depend on the extent to which the main PDT effect is vascular vs cellular, which depends in turn on the photosensitizer and the time interval from its administration to light irradiation.

Alternatively, fluorescence 'reporter' molecules whose spectral or time-decay characteristics are environmentally sensitive, for example to oxygenation or pH (30, 69), could monitor specific tissue factors which are altered by treatment. In general, such forms of immediate tissue response assessment have been little exploited, even qualitatively.

If the observation of autofluorescence photobleaching by Forrer et al (49) is confirmed for different tissue, irradiation wavelengths and physiological states, then, as suggested by them, it could also serve as a photobiological dose metric, and would give additional

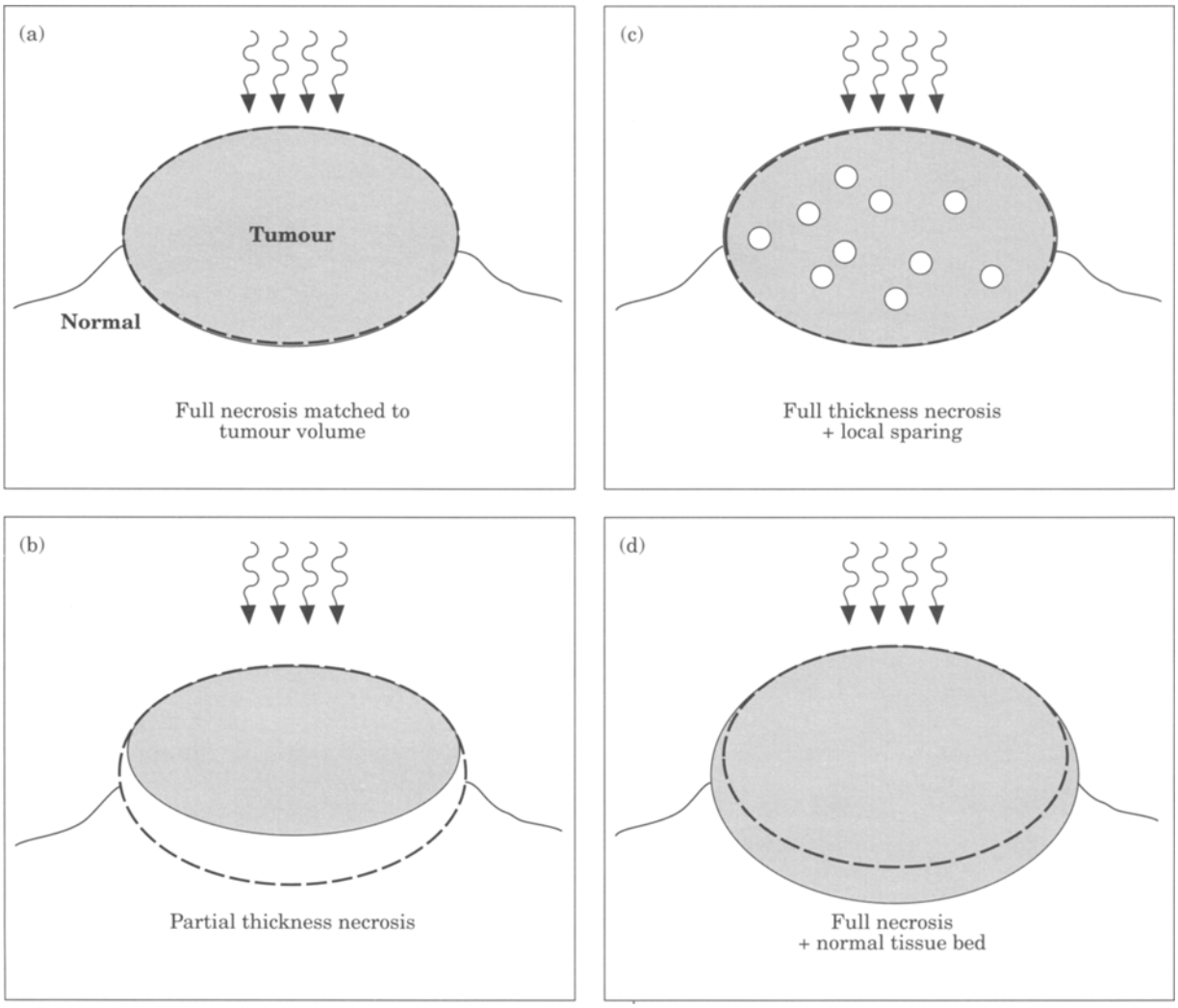


Fig. 8. Schematic illustrations of different tumour response endpoints. The dashed outline indicates the tumour volume and the solid line and shading the zone of effective photodynamic therapy tissue destruction.

information on the ratio of the rate constants for photosensitizer-singlet oxygen vs photosensitizer-biomolecular target interactions. In this way, the endogenous fluorophores would be serving as reporter molecules. For this to be maximally useful, the rate of photosensitizer-fluorophore interactions should have a deterministic and measurable relationship to that for interactions with the targets for photodynamic killing.

TISSUE RESPONSE ENDPOINTS

Finally, it may be necessary to select the dosimetric approach to match the intended or anticipated tissue response endpoint. Figure 8 shows examples of possible response endpoints. In Fig. 8(a,b) the tissue destruction is confined to the tumour volume, within which it is uniform or exceeds some threshold value necessary to cause, for example, complete coagulative necrosis. Clearly, this case is amenable to either explicit or implicit dosimetry methods, and most preclinical in vivo and clinical studies have assumed one or other of these conditions. Surface and/or interstitial measurements of the explicit dose parameters or of the local photobleaching should yield good correlation with these endpoints, and this has been demonstrated for explicit dosimetry in threshold dose studies (31, 32) and by the limited implicit dosimetry studies to date, such as those shown in Fig. 6.

In the case illustrated in Fig. 8(d), the treatment extends to include the tumour bed and, in some transplanted animal tumour models, this has been found necessary in order to achieve complete responses (70), presumably

through shut-down of the vascular supply to the tumour mass. Again, in principle, either dosimetric approach should apply, with the added complication that the photosensitizer uptake, light distribution, tissue oxygenation and intrinsic photodynamic sensitivity will be different in the tumour and normal tissue regions, so that separate, localized measurements in each may be required.

The most difficult case, illustrated in Fig. 8(c), is where there is sparing or incomplete response at localized regions within the target volume. This may be due, for example, to inadequate photosensitizer uptake or local tissue hypoxia, which may be constitutive or PDT-induced. A fundamental problem here is that there is no clear approach to measuring the presence of these focal regions of underresponse, and they may result in predictions based on gross tissue necrosis disagreeing with findings based on tumour regrowth.

CONCLUSIONS

Continuing progress in making PDT a quantitative therapy will depend on developing practical but meaningful measures of PDT 'dose' which will correlate strongly and, ultimately, predictively, with the tissue response and clinical outcome. This paper has presented a framework for the various approaches to PDT dosimetry, and identified some of the important issues and limitations which arise in these. It should be noted that there are few studies to date which have directly investigated critical issues such as the degree of coupling of photosensitizer photobleaching. Hence, those conclusions made above which are necessarily based on specific but limited (and in some cases unpublished) data should be considered as tentative. Nevertheless, they serve well to illustrate the basic concepts.

Fundamental to this framework is the recognition of the interdependency of many of the dosimetric measures, of the real differences between what have been called 'explicit' and 'implicit' dosimetries in this paper, and of the need to tailor the dosimetry to the clinically relevant tissue response endpoints. Untangling these complex relationships will be a major challenge, both conceptually and technically, but is critical to the optimum application of new photodynamic agents and treatment techniques.

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