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**Abstract.** Photodynamic therapy has been considered a potential antimicrobial modality against oral infections, including dental caries. A model to estimate the penetration of both photosensitizers and light through human dentin, a factor of interest in photodynamic therapy, is proposed. The photoacoustic spectroscopy technique was used to evaluate *in vitro* dentin permeability of three different photosensitizers. Using the dentin optical absorption and scattering coefficients, it was possible to propose a semi-quantitative model predicting both photosensitizer and light doses within dentin. The graphic illustrations obtained provided guidelines that may be useful in photodynamic therapy protocols used as antimicrobial tools in caries lesions. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: [10.1117/1.JBO.18.5.055004](https://doi.org/10.1117/1.JBO.18.5.055004)]

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## 1 Introduction

There has been a growing interest in the use of photodynamic therapy (PDT) as an alternative therapy against etiological factors of oral infections, including dental caries and periodontal disease.<sup>1-4</sup> PDT is a treatment characterized by the use of light to activate a photosensitizing agent (PS) in the presence of oxygen, resulting in reactive species *in situ*, which can induce cell death in microorganisms in a process called “antimicrobial photodynamic therapy.”<sup>5</sup>

It is well recognized that PDT has the characteristic of being a local therapy targeting a specific tissue.<sup>3</sup> From this aspect, one of the challenges being faced is to define the appropriate light intensity and PS dose to be used in each specific treatment. This would contribute to the development of an optimized practical protocol to be adopted in dentistry. Thus, knowledge of both light and PS distribution through dentin is essential for dental caries treatment.

The photoacoustic spectroscopy (PAS) technique has been widely used to determine the penetration of formulations through biological tissues.<sup>6-8</sup> This technique has the special characteristic of allowing depth profile studies under clinical conditions, because it demands minimal sample preparation, is a nondestructive method, and is able to allow inspections in highly scattering or optically opaque samples.<sup>9,10</sup> Thus, this method may be useful for measuring the PS penetration rate and its distribution throughout the dentin. The aim of this study was to design a semi-quantitative model that combines the distribution profiles of both PS concentration and light intensity through the dentin and predicts the appropriate PS and light doses to be used in PDT. The tested photosensitizers were

toluidine blue-O (TBO), methylene blue (ME), and malachite green (MG).

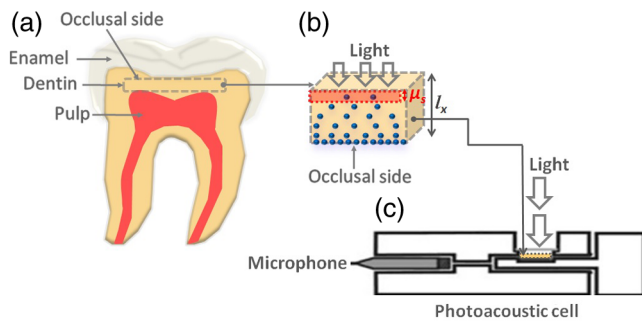
## 2 Materials and Methods

Sound human molar or premolar teeth were collected from patients who needed extraction for orthodontic reasons, in conformity with an informed consent protocol reviewed and approved by the State University of Maringá Ethics Committee on Human Research. After extraction, the teeth were cleaned with gauze and 0.9% physiological solution and then stored in distilled water to prevent dehydration. In order to produce discs of dentin only, the teeth were cut into slices about 0.4 mm thick, using a diamond disc saw. All discs were cut in the transverse direction to that of the dentinal tubules, in the region approximately 2 mm from the cement–enamel junction. From each tooth, a single slice of dentin was obtained and the enamel was removed using sandpaper. The sample dimensions were adjusted to obtain blocks measuring 4 mm (long) × 4 mm (wide) × 0.4 mm (thick). During the PAS measurements, the thicknesses were varied in order to obtain sequential spectra providing the distribution of the PS within the dentin. A total number of 24 dentin blocks were cut, each one having pulp and occlusal dentin surfaces, as shown in Fig. 1. Dentin blocks were kept in saline solution and gently dried before starting the treatment.

The PSs used were diluted in distilled water to concentrations of 0.1 or 0.01 mg/mL. An aliquot of 10  $\mu$ l of the solution containing the PS was applied on the occlusal side of dentin and its interaction was maintained for different time intervals of 1, 5, or 30 min. After that, cotton buds were used to remove the remaining PS from the surface. Next, the samples were placed inside the photoacoustic (PA) cell to take the measurements. This procedure was performed in triplicate.

The PAS measurements were taken with a homemade setup, using the cell illustrated in Fig. 1. Details of the experimental procedure can be found elsewhere.<sup>7,11</sup> The spectra were obtained

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**Fig. 1** Schematic diagram showing the removal of dentin samples (a, b) and their placement on the photoacoustic cell (c).  $\mu_s$  is the thermal diffusion length and  $l_x$  the sample thickness.

between 300 and 800 nm, interval in which the PS presents absorption bands that are of interest to PDT.

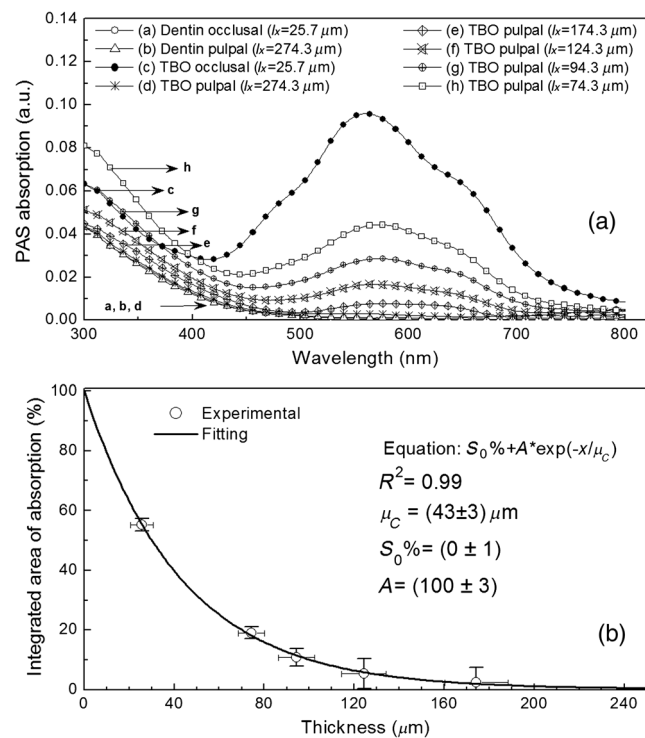
Readouts were taken from the occlusal and pulp surfaces of each sample before and after PS application. For depth profile analysis, treated dentin samples were placed inside the PA cell, with the occlusal side (on which the PS was applied) facing down and the incident light impinging the sample on the pulp side, as illustrated in Fig. 1. In this condition, sequential measurements were made for different thicknesses of each sample, which was obtained by wearing the pulp surface of the tested dentin sample. After this, the spectra were determined as a function of the remaining dentin thickness, from 400 to around 90  $\mu\text{m}$ . Analyses of each spectrum as a function of dentin thicknesses were performed through the integrated areas under the absorption curves, which were considered to be the amount of PS in the corresponding dentin depth.

### 3 Results

The absorption spectra of dentin specimens without PS application were adopted as controls. Figure 2(a) shows the spectra of their occlusal and pulp sides. As expected, the dentin without PS (curves a and b) presents low optical absorption for wavelengths above 450 nm, revealing that the choice of wavelength for PDT is crucial in terms of the target to be attained within the dentin. Next, the readings were performed after PS application. The detection of absorption bands by making the readouts on the pulp side was adopted as a marker of the PS penetration through the dentin. The PS band occurred in the range from 500 to 700 nm.

Figure 2(a), curves d through h, shows the PA spectra of the pulp side as a function of dentin thickness, obtained for the sample exposed to 0.1 mg/mL of TBO for 5 min. The spectra showed a strong increase in the absorption band intensities as the dentin thickness reached the region closer to the occlusal surface where the PS was applied.

In the PA measurements, each spectrum is generated by the contribution of a specific layer of the sample, approximately determined by the thermal diffusion length,  $\mu_s = (D/\pi f)^{1/2}$ , in which  $D$  is the sample thermal diffusivity and  $f$  the light modulation frequency. This parameter is the dimension over which the thermal wave decays to  $1/e$  of its original amplitude and has been used in the analysis as approximately the sampling depth where the PAS signal is generated. The depth of analysis in the sample from the illuminated surface can then be estimated. Using  $D = 0.0025 \text{ cm}^2/\text{s}$  for dentin oriented in the tubular direction<sup>12</sup> and  $f = 16 \text{ Hz}$ ,  $\mu_s$  was estimated to be  $\sim 70 \mu\text{m}$ .



**Fig. 2** (a) Photoacoustic spectra as a function of dentin thickness after toluidine blue-O solution application for 5 min. (b) Integrated area of the absorption bands from Fig. 2(a), fitted to a first-order exponential decay. The value of  $\mu_c$  was  $(43 \pm 3) \mu\text{m}$ .

In other words, when determining the spectrum for a specific thickness with the incident light reaching the pulp side, a layer of about 70  $\mu\text{m}$  contributes to the generation of the PA signal.

Therefore, the variation observed in the absorption band intensities for different thicknesses of the dentin reveals that the PS that has penetrated the dentin is distributed throughout it with some type of gradient profile. The area of each spectrum in Fig. 2(a) was plotted against the dentin thickness as shown in Fig. 2(b). Coordinate  $x$  was plotted with correction made by means of averaging the exponential decay of the thermal diffusion length,  $\bar{\mu}_s$ , for each dentin thickness used, and coordinate  $y$  was normalized in relation to the absorption at the surface, which figures 100%. Amazingly, data in Fig. 2(b) show a typical exponential decay that fits the model  $S(x) = S_0 + Ae^{-x/\mu_c}$ , in which  $S(x)$  represents the normalized PS absorption band areas through the dentin,  $x$  is the relative sample thickness,  $S_0$  is the saturation level for  $x = \infty$ ,  $A$  is the relative amplitude in %, and  $\mu_c$  is the characteristic decay parameter in the  $x$  direction, correlated to the percentage of PS as a function of the dentin thickness. The fitting provided  $\mu_c$  as  $(43 \pm 3) \mu\text{m}$ . This means that at a depth of about 43  $\mu\text{m}$  down from the occlusal surface, the relative attenuation of the amount of PS is 37%. The depth where the amount of PS is reduced to 5% is estimated to be  $x = 3 \mu_c$ , about  $\sim 130 \mu\text{m}$ .

The experiments were repeated in triplicate. For 5- and 30-min periods of contact with the dentin, the average values of  $\mu_c$  were found to be higher for ME with  $3 \mu_c \sim 190 \mu\text{m}$ , while for MG and TBO, they were 144 and 130  $\mu\text{m}$ , respectively. For the 1-min period of contact, the three PSs with concentration of 0.1 mg/mL were detected up to about  $\sim 90 \mu\text{m}$ .

## 4 Discussion

Dentin permeability is known to be a complex process dependent on several chemical and physical conditions.<sup>13–15</sup> In fact, dentin has been considered a semi-permeable membrane, in which fluid movement through its structure is mainly driven by the tubular channels and the nature of the solute/solvent ratio specified by the solute molecular size, geometrical form, and chemical affinity.<sup>14,15</sup> The permeation of different solutions into dentin has been studied; however, no model was found that could predict a PS and light permeation profile and its concentration throughout the diffusion process.

In dentistry, it is important to know the depth of permeation of the PS and the light used in PDT to ensure that they reach the full extent of decayed tissue to promote the desired therapeutic effect. Furthermore, an excess of dye could stain sound tooth structure or promote undesirable effects on pulp tissue. Thus, the proposed model could help the adjustment of both PS concentration and light intensity in clinical practice.

The present study with PAS revealed that the tested PS showed permeability through dentin, with depth profiling followed by a single exponential decay dependence. For example, for MB, the value of  $3 \mu_c$  was found to be about  $190 \mu\text{m}$ . This result indicates that the procedure adopted allows the distribution profile of the PS through the dentin to be obtained. In a previous study, *Streptococcus* was detected in dentin at a maximum depth of about  $200 \mu\text{m}$ .<sup>16</sup> Therefore, the results of the present study show that the tested PSs can reach the infected region that requires treatment.

However, it is important to bear in mind that in order to inactivate the microorganisms, it is necessary for the light to be transmitted through the dentin and activate the PS. Thus, the

next step will be used for the development of a semi-quantitative model to predict both PS concentration and incident light intensity as a function of dentin thickness.

First, as described by Manly et al.,<sup>17</sup> the transmittance of light through dentin, which is subject to great interference from light scattering, can be evaluated by

$$\frac{I(t)}{I_0} = (Pe^{\lambda t} + Qe^{-\lambda t})^{-1}, \quad (1)$$

in which  $I_0$  and  $I(t)$  represent the intensities of the incident and transmitted beam;  $t$  is a parameter describing the dimension crossed by the light beam ( $x$  or  $y$ );  $P$ ,  $Q$ , and  $\lambda$  are parameters written in terms of the scattering coefficient ( $s$ ) and absorption coefficient ( $k$ ) as

$$P = \frac{(\lambda + k)^2}{4\lambda k}, \quad (2)$$

$$Q = -\frac{(\lambda - k)^2}{4\lambda k}, \quad (3)$$

$$\lambda = \sqrt{k(k + s)}. \quad (4)$$

Note that  $I_0 = P + Q = 1$ . These equations were used as the basis for the intended model. First, Eqs. (2), (3), and (4) were substituted in Eq. (1) in order to represent  $I(t)$  as a function of the coefficients of absorption ( $k$ ) and scattering ( $s$ ). Thus, the intensity of the transmitted light beam  $I(t)$  through the dentin can be written as

$$I(t) = \frac{2\sqrt{k(k + s)}}{2\sqrt{k(k + s)} \cosh[t\sqrt{k(k + s)}] + (2k + s) \sinh[t\sqrt{k(k + s)}]}. \quad (5)$$

In the PDT procedure, PS is applied on the dentin; thus light transmittance is also attenuated by its optical absorption. Then, to adapt Eq. (5), the optical absorption coefficient of the PS should be considered. To do so, it is necessary to bear in mind the orientation of both the PS concentration gradient and light illumination, as shown in Fig. 3(a). PS application in the longitudinal direction ( $x$  axis) was adopted, and the incident light was applied in both the longitudinal ( $x$ ) and transverse ( $y$ ) directions. Under these conditions, the model was developed considering two different situations for the direction of illumination.

The case of transverse illumination ( $t = y$ ) is represented in Fig. 3(a). The absorption coefficient ( $k$ ) of Eq. (5) is redefined since it varies for different ranges of  $y$ . Thus,  $k$  can be defined as follows:

$$k = \begin{cases} k_d + k_1, & 0 \leq y \leq Y_0 \\ k_d + k_1(Y_0) + k_2, & Y_0 \leq y \leq l_s \end{cases}, \quad (6)$$

in which

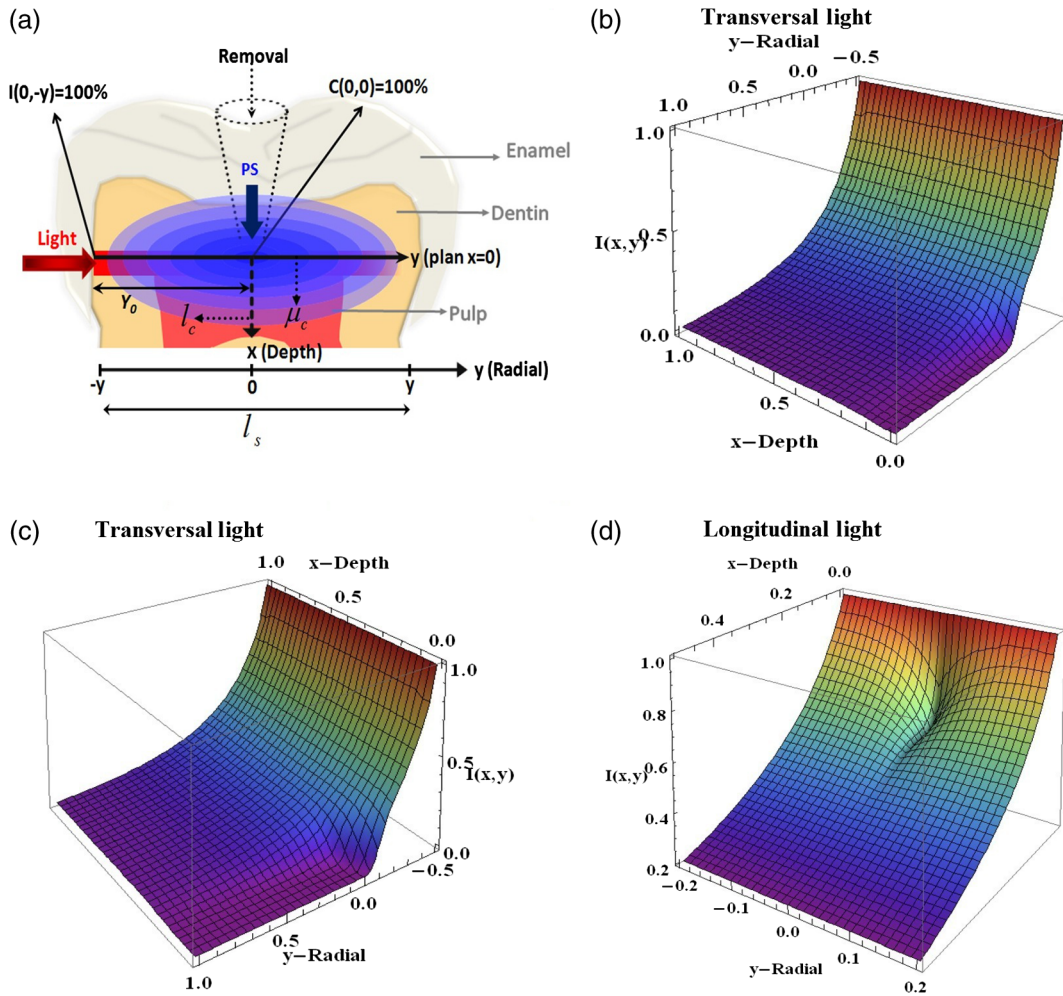
$$k_1 = k_0 \exp\left\{-\left[\left(\frac{x}{\mu_c}\right) + \frac{|Y_0 - y|}{l_c}\right]\right\}, \quad (7)$$

$$k_2 = k_0 \left( \exp\left[\frac{-x}{\mu_c}\right] \left\{ 1 - \exp\left[\frac{-(y - Y_0)}{l_c}\right] \right\} \right). \quad (8)$$

The parameter  $k_1$  represents the exponential increasing of the optical absorption coefficient along the  $y$  axis in the interval  $0 \leq y \leq Y_0$ . The parameter  $k_2$  was defined as the  $y$ -mirrored form of Eq. (7) to describe the decreasing of the absorption coefficient in the region  $Y_0 \leq y \leq l_s$  in addition to  $k_1(y = Y_0)$ . Both  $k_1$  and  $k_2$  decrease with the same exponential dependence along the  $x$  axis. These definitions are illustrated in Fig. 3(a).

The parameters  $k_d$  and  $s$  are the coefficients of absorption and scattering of dentin, respectively,  $k_0$  is the absorption coefficient of the PS and  $l_c$  the characteristic penetration depth of the PS in the  $y$  direction. In this work, we considered an approximately isotropic PS concentration distribution,  $l_c \sim \mu_c$ . As adopted in practical protocols, the figures were constructed illustrating application of the photosensitizers on dentin after part of the dental tissue was removed. Thus,  $Y_0$  corresponds to the central position of the hole where the PS is applied and  $l_s$  is the sample thickness in the  $y$  direction. The position  $y = Y_0$  corresponds to the region with the highest concentration





**Fig. 3** (a) Picture representing a tooth after removal of part of the dental tissue and possible geometry of the incident light. (b) Simulation with Eq. (11) using the absorption coefficient of  $2.8 \text{ mm}^{-1}$  and with transversal illumination ( $y$  axis). (c) Simulation with Eq. (11) using the absorption coefficient of  $28 \text{ mm}^{-1}$  and with transversal illumination. (d) Simulation with Eq. (11) using the absorption coefficient of  $28 \text{ mm}^{-1}$  and with longitudinal illumination ( $x$  axis). The value used for  $Y_0$  was  $0.5 \text{ mm}$  in (b) and (c) and  $0.2 \text{ mm}$  in (d).

of the dye. The simulations were performed considering  $Y_0 = 0.5 \text{ mm}$ .

From Eqs. (7) and (8) it is possible to see that  $k_0$  values presented exponential decays in  $x$  and  $y$  directions, revealing how the PS diffuses through the dentin.

Substituting Eqs. (7) and (8) according to the boundaries in Eq. (6), the transmitted intensity of light through the dentin with PS can be obtained. For transverse illumination,  $k$  is replaced by  $y$  in Eq. (5). This means that the transmitted beam is now written in terms of the dependence in both  $y$  and  $x$  directions,

$$I(x, y) = \frac{2\sqrt{k(k+s)}}{2\sqrt{k(k+s)} \cosh[y\sqrt{k(k+s)}] + (2k+s) \sinh[y\sqrt{k(k+s)}]}. \quad (9)$$

First, the simulations were performed as a function of  $x$  and  $y$  for the condition in which the PS was applied in the  $x$  and the illumination in the  $y$  direction. Two cases were studied: the first with the absorption coefficient of the PS ( $k_0$ ) at the concentration used in the photoacoustic experiments,  $2.8 \text{ mm}^{-1}$ , and the second with its value 10 times higher. The dentin absorption and scattering coefficients were taken from the literature, at  $615 \text{ nm}$ .<sup>18</sup> The value of used was  $0.5 \text{ mm}$ . The results are shown in Figs. 3(b) and 3(c). There are attenuations of the light intensities in the  $y$  direction reaching a minimum at  $x = 0$ , the surface where PS was applied. When  $y < Y_0$ , the light is transmitted through the

dentin only. As approaches  $Y_0$ , it passes through the regions with PS, with significant attenuation of the beam near the region, where the PS concentration is maximum. For  $y > Y_0$ , the light intensity decay is slow.

For illumination in the longitudinal direction ( $t = x$ ),  $t$  was changed to  $x$  in Eq. (5). In this case, the light passes through the region where the PS was applied. The condition for absorption coefficient ( $k$ ) is now given by

$$k = k_d + k_0 \left[ \exp\left(\frac{-x}{\mu_c}\right) \exp\left(\frac{-|y - Y_0|}{l_c}\right) \right], \quad (10)$$

**Table 1** Correlation between light transmittance  $I(x, y)\%$  and PS concentration  $C(x, y)\%$  for different depth  $x$  and radial position  $y$ , using transversal illumination. The maxima are at  $C(0, 0) = 100\%$  and at  $I(0, -y) = 100\%$ .

$x$ (Depth) (mm)	$y$ (Radial) (mm)									
	-0.5		-0.25		0		0.25		0.5	
	$I(\%)$	$C(\%)$	$I(\%)$	$C(\%)$	$I(\%)$	$C(\%)$	$I(\%)$	$C(\%)$	$I(\%)$	$C(\%)$
<b>0</b>	100	0	41	0.3	4	100	0.8	0.3	0.007	0
<b>0.05</b>	100	0	41	0.09	12	31	2	0.09	0.5	0
<b>0.1</b>	100	0	41	0.03	18	10	6	0.03	3	0
<b>0.25</b>	100	0	41	0	21	0.3	11	0	6	0
<b>0.5</b>	100	0	41	0	21	0	11	0	6	0
<b>0.75</b>	100	0	41	0	21	0	11	0	6	0
<b>1</b>	100	0	41	0	21	0	11	0	6	0

$$I(x, y) = \frac{2\sqrt{k(k+s)}}{2\sqrt{k(k+s)} \cosh[x\sqrt{k(k+s)}] + (2k+s) \sinh[x\sqrt{k(k+s)}]}. \quad (11)$$

Here, the absorption coefficient is maximum at  $y = Y_0$ . The simulation with this equation using  $k_0 = 28 \text{ mm}^{-1}$  and  $Y_0 = 0.2 \text{ mm}$  is shown in Fig. 3(d).

The contour maps provide a study protocol predicting the doses of both PS and light to be used in the PDT. For better illustration, Table 1 presents the correlation between light transmittance  $I(x, y)\%$  and PS concentration  $C(x, y)\%$  for different depth,  $x$ , and radial position,  $y$ , that was generated by considering the case of transversal illumination. Maxima are at  $C(0, 0) = 100\%$  and at  $I(0, -y) = 100\%$ .

Although sound dentin blocks were kept in saline solution, dentinal fluid flow produced by intrapulpal pressure or demineralized tissue were not taken into account during the analysis. In addition, the dentinal smear layer was not removed to test PS permeability. As a final remark, it should be stressed that the presented model was built to predict different PS and light diffusion behaviors, that combined with the knowledge of adequate dose to inactivate microorganisms, may allow avoiding of overdose of both PS and light intensity, contributing to patient safety.

## 5 Conclusion

In conclusion, a study protocol for PDT allowing appropriate choice of both photosensitizers and light doses penetrating through dentin was proposed. The model was developed using the PAS technique that provided *in vitro* dentin permeability of the tested photosensitizers. The contour maps provided guidelines for appropriate choice of photosensitizer and light doses to be used in PDT, contributing to the effort to establish a clinical protocol for dental caries treatment using PDT.

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