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# Antimicrobial Photodynamic Action on Dentin Using a Light-Emitting Diode Light Source

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## Abstract

***Objective:*** The aim of this study was the evaluation of two different photosensitizers activated by red light emitted by light-emitting diodes (LEDs) in the decontamination of carious bovine dentin.
***Materials and Methods:*** Fifteen bovine incisors were used to obtain dentin samples which were immersed in brain-heart infusion culture medium supplemented with 1% glucose, 2% sucrose, and 1% young primary culture of *Lactobacillus acidophilus* $10^8$ CFU/mL and *Streptococcus mutans* $10^8$ CFU/mL for caries induction. Three different concentrations of the Photogem solution, a hematoporphyrin derivative (1, 2, and 3 mg/mL) and two different concentrations of toluidine blue O (TBO), a basic dye (0.025 and 0.1 mg/mL) were used. To activate the photosensitizers two different light exposure times were used: 60 sec and 120 sec, corresponding respectively to the doses of 24 J/cm$^2$ and 48 J/cm$^2$.
***Results:*** After counting the numbers of CFU per milligram of carious dentin, we observed that the use of LED energy in association with Photogem or TBO was effective for bacterial reduction in carious dentin, and that the greatest effect on *S. mutans* and *L. acidophilus* was obtained with TBO at 0.1 mg/mL and a dose of 48 J/cm$^2$. It was also observed that the overall toxicity of TBO was higher than that of Photogem, and that the phototoxicity of TBO was higher than that of Photogem.
***Conclusion:*** Based on our data we propose a mathematical model for the photodynamic effect when different photosensitizer concentrations and light doses are used.

## Introduction

PHOTODYNAMIC THERAPY (PDT) is a technique that involves the activation of certain dyes (photosensitizers) by light in the presence of tissue oxygen, resulting in the production of reactive radicals capable of inducing cell death. Until recently, the main application of PDT was to treat malignant and some benign lesions;[1,2] however, the efficacy of this procedure for microbial reduction has been demonstrated in several studies.[3–6] This emerging class of applications, which we refer to as antimicrobial photodynamic action (APDA), shows great potential.[7–10]

Currently, lasers are the most common light sources used to activate the photosensitizers. However, the development of bright light-emitting diodes (LEDs) provides an alternative light source for PDT.[11–13] The light produced by an LED has characteristics different from those of laser light: it is a narrow-band non-coherent type of energy that is not diffraction-limited. In this respect it is similar to the light of a suitably filtered mercury lamp. LED devices have, in general, a lower cost and simpler technology compared to other laser devices, and moreover, they can be readily assembled in several configurations that are suitable for different types of anatomical illumination.

Studies from the 1990s have shown that a large number of types of oral bacteria, including periodontal pathogenic and cariogenic bacteria, are susceptible to photodynamic therapy.[14–18] However, the use of LED-based light sources to activate photosensitizers for use against oral microorganisms is a recent development. Study of their use is important to promote the clinical use of LEDs instead of lasers for therapeutic applications in dentistry.[19]

Our study evaluated the efficiency of APDA using two different photosensitizers activated by LED energy for de-

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contaminating carious bovine dentin. The investigation of the decontamination induced in a tissue environment is relevant since the photosensitizer's distribution and its interaction with light will vary depending on the culture medium.

## Materials and Methods

Fifteen bovine incisors without any visible structural anomalies were used in this study. The periodontal tissue was scaled and the buccal enamel was removed using a conical diamond bur in a high-speed handpiece. From each tooth, four dentin fragments were obtained using a titanium trephine drill. Each dentin fragment was air-dried for 60 sec and weighed; the average weight was 23 mg. The fragments were autoclaved for 20 min at 121°C and transferred to individual test tubes, each containing 2 mL of a brain-heart infusion (BHI) culture medium 1 g/100 mL supplemented with glucose and 2 g/100 mL sucrose PA (Pro-analysis). For each 50 mL of medium solution, 5 mL of $10^8$ CFU/mL *Lactobacillus acidophilus* (ATCC #ITAL-523) and 5 mL of $10^8$ CFU/mL *Streptococcus mutans* (ATCC #25175) were added to induce bacterial colonization. The test tubes were maintained in a micro-aerobic environment at 37°C for 14 d, with the solution changed every 48 h. Following the 14-day period, the specimens were maintained under refrigeration at 4°C until treatment.

The specimens of the experimental groups were washed in sterile saline solution and then immersed for 60 sec in a solution containing either Photogem® (Photogem, Moscow, Russia), a hematoporphyrin derivative, or toluidine blue O (TBO) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), a basic dye. Three different concentrations of Photogem solution, 1 mg/mL, 2 mg/mL, and 3 mg/mL, and two different concentrations of TBO, 0.025 mg/mL and 0.1 mg/mL, were used. These solutions were prepared with distilled water at pH values of 7.4 for Photogem and 5.1 for TBO, as determined in previous studies using these photosensitizers.[13,20]

A homemade light source based on an LED emitter (LXHL-PD01; Luxeon, San Jose, CA, USA) for photosensitizer activation was developed. The generated light was optically collimated to a spot size 0.8 cm in diameter. The total irradiance was 400 mW/cm$^2$ from an output power of 200 mW. The wavelength was centered at 630 nm with a bandwidth of ± 10 nm. To keep the semiconductor junction temperature below 60°C, the emitter was mounted on a heat sink constructed of a metallic base as shown in Fig. 1. Two fluence levels were investigated, 24 J/cm$^2$ and 48 J/cm$^2$, with exposure times of 60 sec and 120 sec, respectively.

In the control group, after the caries induction and without any treatment, the specimens were placed in 5 mL of saline solution. The individual action of the photosensitizer (Photogem or TBO) and of illumination was investigated at all experimental parameters. There were experimental groups using Photogem or TBO under different concentrations and no light exposure, and some non-sensitized specimens were illuminated with both fluences. For each group three samples were evaluated.

After treatment the specimens were placed into individual test tubes with 5 mL of saline solution and stirred in a vortexer (60 Hz; Heidolph, Kelheim, Germany) for 60 sec. From this solution, an aliquot of 0.5 mL was transferred to another test tube with 4.5 mL of saline solution, followed by two similar serial dilutions; then 0.1 mL of each solution was put into Petri dishes containing modified BHI culture medium with agar, after which the plates were incubated micro-aerobically at 37°C for 48 h.

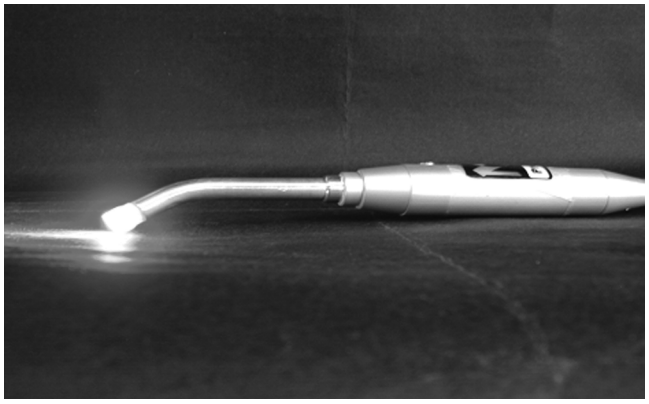

**FIG. 1.** The LED device used in our study.

The number of bacterial colony-forming units (CFU) was counted using a digital counter (Gallenkamp, Leicestershire, UK). The number of CFU was divided by the weight of each sample to obtain the number of CFU per milligram (CFU/mg) of the carious dentin. In order to compare the results between the different photosensitizer concentrations, the measurements were normalized to the CFU/mg of the control group (non-sensitized and non-illuminated) and was defined as the survival factor (SF). An SF of 1 means that 100% of the colonies survived the treatment, and an SF of 0 represents complete elimination of the colonies. We calculated the percentage killed using a simple equation: % killed = (1– SF) × 100%.

We developed an empirical expression for the variations in SF for different Photogem and TBO concentrations (C) and fluences (D). The complexity of a biological system such as this makes establishment of a mathematical relation to allow quantification, comparison, and extrapolation of the observations was difficult. This relation, besides being empirical, has features that may assure its validity within certain limits.

The SF variation in the absence of light corresponds to the dark toxicity of the two photosensitizers at each concentration. Our expression may present a solution within a range from 0 to 1. In samples in which no photosensitizer was used (C = 0) complete survival (SF = 1) is expected. On the other hand, even if the photosensitizer has a very low dark toxicity, a concentration approaching infinity must result in complete elimination of the colonies (SF = 0). These two limits are incorporated into equation [1], for the dependence of SF upon the drug concentration when no light is present.

$$SF(D = 0) = \frac{1}{1 + \frac{C}{C_0}} \qquad [1]$$

For very small $C/C_0$, this expression can be mathematically expanded, resulting in SF (D = 0) = $1 - C/C_0$, which implies a linear toxicity effect proportional to the concentration used.

Here D is the fluence (in this case, zero), C is the concentration expressed in milligrams per milliliter, and $C_0$ is a con-

stant related to the dark toxicity of the photosensitizer. The value of $C_0$ can be determined through a mathematical adjustment of equation [1] using experimental data for each photosensitizer used. $C_0$ is expected to depend intrinsically upon the photosensitizer.

Since the presence of light amplifies the toxic effect of the drug, we expect the toxicity to increase with increasing light dose. We added a term to equation [1] that represents the enhancing effect of the light, resulting in equation [2]:

$$SF(D,C) = \frac{1}{1 + \frac{C}{C_0} G\left(\frac{D}{D_0}\right)} \quad [2]$$

Here the function $G(D/D_0)$ must have the property of increasing value with D, and having the value of unity at D = O. The simplest function that can be represented is $G(D/D_0) = 1 + D/D_0$, where $D_0$ is a constant related to the phototoxicity, and it was again determined by a linear regression fitting the experimental data. A higher value means that a higher light dose is necessary to kill the microorganisms. Alternatively, lower values of $D_0$ imply a stronger effect of light in the APDA application. So, finally another expression can be defined, equation [3]:

$$SF(D,C) = \frac{1}{1 + \frac{C}{C_0}\left(1 + \frac{D}{D_0}\right)} \quad [3]$$

## Results

The results obtained for the SF are shown in Fig. 2, where each data point is the average of three specimens. The error bars correspond to the standard deviation of the data from the average. The solid lines correspond to the best adjustment for the data using equation [2]. Fig. 2A and D (dark toxicity) show that the concentrations of photosensitizers used produced toxicity at different levels. Equation [2] can be adjusted to the experimental data resulting from D = 0, to $C_0 \approx 0.845$ *mg/ml* for Photogem and $C_0 \approx 0.127$ *mg/ml* for TBO. This indicates that TBO has a higher dark toxicity. Exposure to LED light at the dose of 24 J/cm$^2$ resulted in a smaller SF, as shown in Fig. 2B and E. Taking into consideration the value of $C_0$, equation [2] can be compared with the data, and the best agreement is obtained for $D_0 \approx 9.923$ *J/cm*$^2$ for Photogem and $D_0 \approx 2.081$ *J/cm*$^2$ for TBO. Since higher values of $D_0$ mean lower phototoxicity, TBO shows greater phototoxicity than Photogem.

It is possible to generate from equation [2] plots relating the concentration of Photogem or TBO to the light dose needed to achieve different levels of SF (Fig. 3A and B). The plots are divided into regions separated by various curves. Region A1 corresponds to the light dose and drug concentration required to produce a SF <0.01; A2 yields $0.01 < SF < 0.05$; A3 yields $0.05 < SF < 0.1$, and A4 yields $0.1 < SF < 1$. Using these plots, one can determine the proper combination of photosensitizer concentration and light dose to kill 90%, 95%, or 99% of the microorganisms, corresponding to SF values of 0.1, 0.05, or 0.01, respectively.

The solid lines in Fig. 2 correspond to the best mathematical adjustment using equation [3], to obtain optimal values of $C_0$ and $D_0$ for each photosensitizer tested.

## Discussion

Photodynamic action has been used to kill oral microorganisms since the beginning of the 1990s, when studies demonstrated that some photosensitizers show an affinity for bacterial walls and can be photoactivated to cause the desired damage.[21–23] Excited photosensitizer molecules can transfer energy to nearby molecules, resulting in the formation of reactive molecules as singlet oxygen, superoxide, and other free radicals, capable of causing damage and even death of cells and bacteria.[5,7,24,25]

Ito, in 1977,[26] showed that TBO was photodynamically active, promoting yeast cell death without inducing any genetic alterations, and that the cellular membrane was the reaction site for singlet oxygen. The effect on the yeast cells was to increase permeability and loss of control over permeability, resulting in an imbalance of intracellular substances, resulting in cellular death without any apparent chromosomal damage. In 1995, Paardekooper et al.[27] observed that toluidine blue entered into cells during illumination because of a sudden change in the cellular membrane, resulting in damage to the cellular membrane and intracellular structures.

Studies of biofilms[15] have shown the efficacy of TBO at concentrations of 0.01% and 0.1% when illuminated with HeNe laser energy, for reduction of the numbers of *Streptococcus sanguis, Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum*. An apparent bactericidal effect was observed after exposure to laser energy for 30 sec, with a light dose of 219 mJ and fluence of 16.5 J/cm$^2$. Burns et al.,[28] in 1993, observed that when a suspension of cariogenic bacteria (*Streptococcus mutans, S. sobrinus, Lactobacillus casei* and *Actinomyces viscosus*) was mixed with TBO at 50 $\mu$g/mL and exposed to 7.3 mW of HeNe laser energy, a considerable amount of cellular death was accomplished with a fluence of 33.6 J/cm$^2$. In the case of TBO, the results of our study are similar to those of Burns et al.[28] If equation [2] is used with $C_0$ and $D_0$ values determined for TBO from our data (0.13 mg/mL and 9 J/cm$^2$, respectively), then C $\approx$ 0.05 mg/mL and D = 34 J/cm$^2$, and an SF of $5.6 \times 10^{-2}$ is obtained for *S. mutans*. This is a much lower value than the reduction of viability observed by Burns et al.[28] This difference arises mainly from intrinsic differences between the two light sources used. While Burns et al. used 500 mW/cm$^2$ of light centered at 632 nm,[28] our experiment utilized approximately 400 mW/cm$^2$ distributed in a range from 615–638 nm, covering a much broader spectral band, and possibly influencing the resulting bactericidal effect. Okamoto et al.[16] also reported the death of various species of *Streptococcus* using a HeNe laser dose of 720 mJ and a fluence of 5.7 J/cm$^2$ with TBO at 7.5 $\mu$g/mL.

In 2005 Zanin et al.[19] evaluated the antimicrobial effect of TBO in combination with either a HeNe laser or a LED, on the viability and architecture of *Streptococcus mutans* biofilms and observed that the bactericidal effect was light dose-dependent, an effect we also observed in this study. The reductions in viability up to 99.99% with both light sources were observed using energy densities between 49 and 294 J/cm$^2$, a pre-irradiation time of 5 min, and TBO concentration of 100 mg/L.

Other studies, including the one developed by Venezio et al. in 1985,[29] previously demonstrated that hematoporphyrin

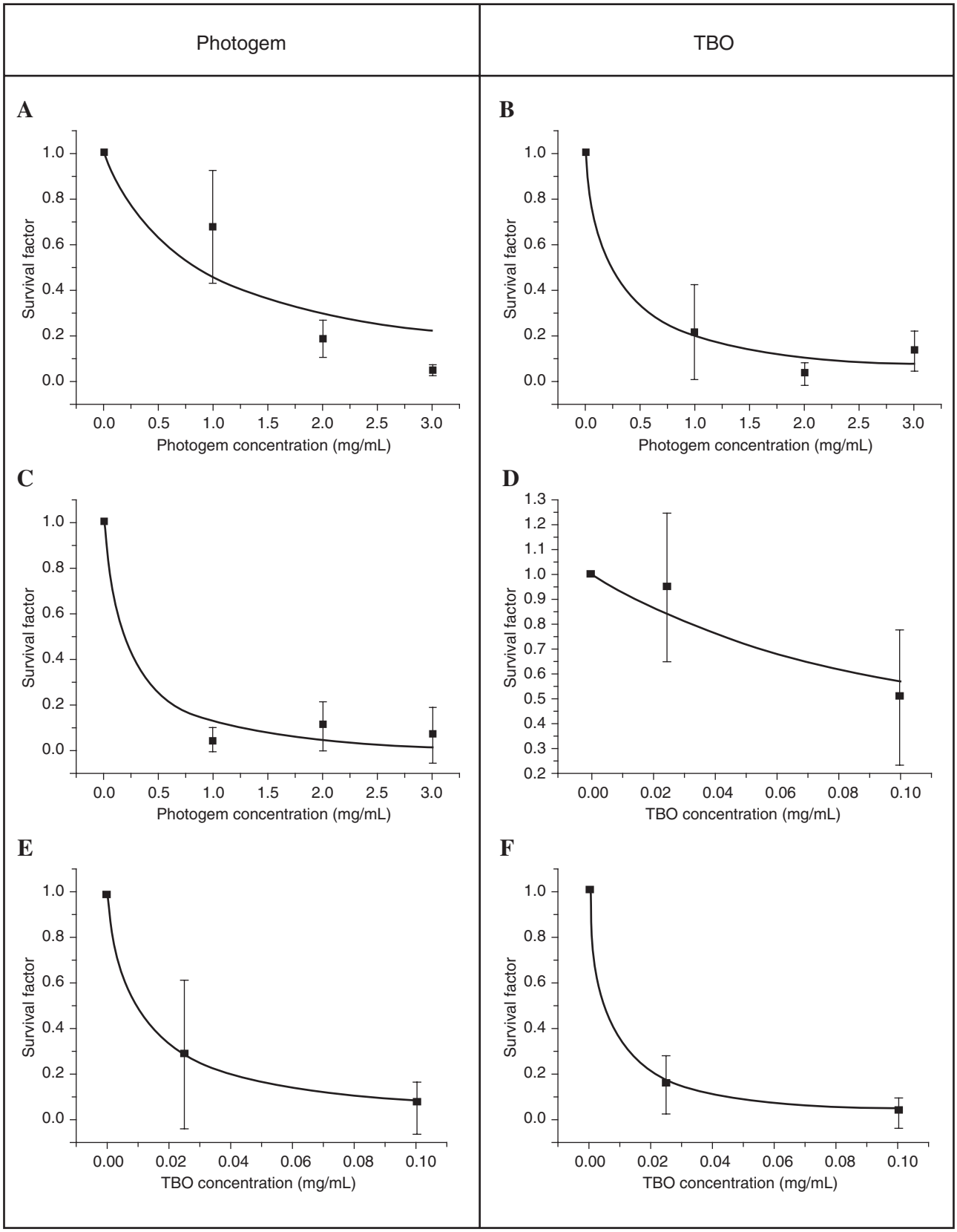


**FIG. 2.** (**A** and **D**) Dark toxicity of the photosensitizers. APDA results as evaluated by SF for 24 J/cm$^2$ (**B** and **E**) and 48 J/cm$^2$ (**C** and **F**) of laser irradiation.

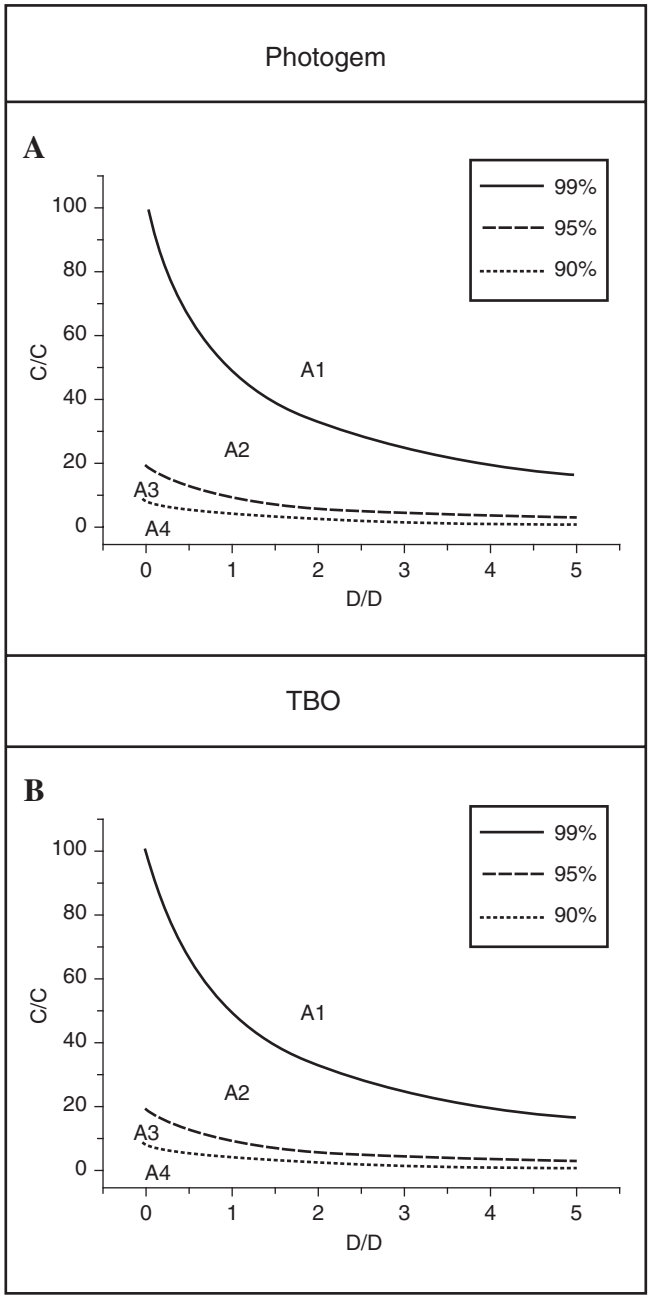


**FIG. 3.** Correlation between relative parameters of (**A**) Photogem and (**B**) TBO concentration and light dose to reduce SF.

derivatives show a bactericidal effect on *S. mutans* and other microorganisms.[20,24,30] Additionally, hematoporphyrin derivatives were the first drugs authorized by the U.S. Food and Drug Administration for clinical use in PDT.[7] Their cytotoxic effect is mainly carried out by the production of singlet oxygen. Gram-negative bacteria seem to be more resistant to this treatment, probably due to their more complex cell wall.[26,31]

Figures 2A and D show the dark toxicity of Photogem and TBO. The toxicity of both photosensitizers in the absence of light increased with increasing concentration, but the effect is more pronounced for TBO. Figures 2B, E, C, and F show the phototoxicity of the photosensitizers activated with LED light with doses of 24 and 48 J/cm$^2$. These graphs also indicate a higher phototoxicity of TBO. Malik et al.[24] and Dobson and Wilson,[15] using hematoporphyrin at 0.005% and a HeNe laser emitting 7.3 mW at 5.5 J/cm$^2$, did not report any favorable results for gram-negative microorganisms, although Wilson et al.[22] noticed lethal photosensitization of *P. gingivalis* with hematoporphyrin when these microorganisms were in suspension, but not in biofilms.

Many studies have shown TBO's effectiveness as a photosensitizer, but were performed with bacteria in suspension.[5,8,13,16,17,22] However, similar studies demonstrated that the reduction of *S. mutans* in carious dentin was less than that seen in suspension, and was also less than that attained in a collagen matrix. In that study, it was concluded that the time of contact between the bacteria and TBO was a critical factor.[32]

In 2003, Williams et al.[13] noted 100% death of *S. mutans* in a plankton suspension, using a diode laser emitting at 633 $\pm$ 2 nm with fluences ranging from 0.4–4.8 J with TBO as the photosensitizer. In 2004 the same authors[32] using the same light source, with fluences varying from 1.8–14.4 J and TBO at 10 $\mu$g/mL obtained a reduction of *S. mutans* in a collagen matrix and carious human dentin, although the dentin results were more variable than those observed in the collagen matrix. Those results are similar to the results found in our study, although the presence of the biofilm on the dentin itself negatively affect the efficacy of the technique, because the success of the process depends on photosensitizer diffusion throughout the dentin, and on light penetration and scattering in the tissue.

We believe that an important contribution of our study is the use of the empirical mathematical equation [2] relating the light dose, photosensitizer concentration, and bactericidal effect. Our experimental results indicate that there was bacterial reduction at all investigated parameters using the photosensitizers Photogem and TBO for treatment of S. *mutans* and *L. acidophilus*. Equation [2] allows the extrapolation of our results to parameters not yet investigated. In Fig. 3A and B we plot the SF determined by equation [2] as a function of light dose for different concentrations of Photogem and TBO. We note the general effect that toxicity, with and without exposure to light, increases with increasing concentration of photosensitizer. In addition, it is apparent that light alone, without any photosensitizer, has no bactericidal effect. The effect for a large range of parameters is more evident in Fig. 2A and B, where we can readily determine the combination of light dosage and photosensitizer concentration to achieve the desired level of bacterial reduction.

## Conclusion

In conclusion, we have shown that the use of LED light in association with Photogem or TBO was effective for bacterial reduction of *S. mutans* and *L. acidophilus* in carious dentin. The greatest toxic photodynamic effect was obtained with TBO at 0.1 mg/mL and 48 J/cm$^2$ of illumination. It was also observed that the dark toxicity of TBO is higher than of Photogem. Similarly, the phototoxicity of TBO is higher than that of Photogem.

We used an empirical mathematical expression to correlate the measured bactericidal effect with drug concentration and fluence. The use of such a formula provides a great deal of data about the expected results for a large variety of parameters.

This expression may represent a simple tool to evaluate the efficacy of APDA for different microorganism species, and this concept may be used in the dosimetry planning of future antimicrobial clinical applications. The introduction of constants that quantify dark toxicity and phototoxicity is a good way to compare different photosensitizers operating under equivalent conditions.

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## References

1. Al-Watban, F.A., and Zhang, X.Y. (2005). Photodynamic therapy of human undifferentiated thyroid carcinoma-bearing nude mice using topical 5-aminolevulinic acid. Photomed. Laser Surg. 23, 206–211.
2. Tardivo, J.P., Sel Goglio, A., Pachoal, L.H., and Baptista, M.S. (2006). New photodynamic therapy protocol to treat AIDS-related Kaposi's sarcoma. Photomed. Laser Surg. 24, 528–531.
3. Bedwell, J., Holton, J., Vaira, D., Macrobert, A.J., and Bown, S.G. (1990). *In vitro* killing of *Helicobacter pylori* with photodynamic therapy. Lancet. 335, 1287.
4. Wilson, M. (1994). Bactericidal effect of laser light and its potential use in the treatment of plaque-related diseases. Int. Dent. J. 44, 181–189.
5. Sibata, C.H., Colussi, V.C., Oleinick, N.L., and Kinsella, T.J. (2000). Photodynamic therapy: a new concept in medical treatment. Braz. J. Med. Biol. Res. 33, 869–880.
6. Smucler, R., and Jatsová, E. (2005). Comparative study of a aminolevulic acid photodynamic therapy plus pulsed dye laser versus pulsed dye laser alone in treatment of viral warts. Photomed. Laser Surg. 23, 202–205.
7. Wainwright, M. (1998). Photodynamic antimicrobial chemotherapy (PACT). J. Antimicrob. Chemother. 42, 13–18.
8. Qin, Y., Luan, X., Bi, L., He, G., Bsai, X., Zhou, C., and Zhang, Z. (2008). Toluidine blue-mediated photoinactivations of periodontal pathogens from supragengival plaques. Lasers Med. Sci. 23, 49–54.
9. Oliveira, R.R., Schwartz-Filho, H.O., Novaes, A.B. Jr., and Taba, M. Jr. (2007). Antimicrobial photodynamic therapy in the non-surgical treatment of aggressive periodontitis: a preliminary randomized controlled clinical study. J. Periodontol. 78, 965–973.
10. Munin, E., Giroldo, L.M., Alves, L.P., and Costa, M.S. (2007). Study of germ tube formation by *Candida albicans* after photodynamic antimicrobial chemotherapy (PACT). J Photochem. Photobiol. B. 88, 16–20.
11. Lui, H., Hoobs, L., Tope, W.D., et al. (2004). Photodynamic therapy of multiple nonmelanoma skin cancers with verteporfin and light-emitting diodes. Arch. Dermatol. 140, 26– 32.
12. Yang, C.H., Lee, J.C., Chen, C.H., Hui, C.Y., Hong, H.S., and Kuo, H.W. (2003). Photodynamic therapy for bowenoid papulosis using a novel incoherent light-emitting diode device. Br. J. Dermatol 149, 1292–1308.
13. Williams, J.A., Pearson, G.J., Colles, M.J., and Wilson, M. (2003). The effect of variable energy input from a novel light source on the photoactivated bactericidal action of toluidine blue O on *Streptococcus mutans*. Caries Res. 37, 190–193.
14. Burns, T., Wilson, M., and Pearson, G.J. (1995). Effect of dentine and collagen on the lethal photosensitization of *Streptococcus mutans*. Caries Res. 29, 192–197.
15. Dobson, J., and Wilson, M. (1992). Sensitization of oral bacteria in biofilms to killing by light from low-power laser. Arch. Oral. Biol. 37, 883–887.
16. Okamoto, H., Iwase, T., and Morioka, T. (1992). Dye-mediated bactericidal effect of He-Ne laser irradiation on oral microorganisms. Lasers Surg. Med. 12, 450–458.
17. Sarkar, S., and Wilson, M. (1993). Lethal photosensitization of bacteria in subgingival plaque from patients with chronic periodontitis. J. Periodontal Res. 28, 204–210.
18. Soukos, S., Wilson, M., Burns, T., and Speigh, P.M. (1996). Photodynamic effects of toluidine blue on human oral keratinocytes and fibroblasts and *Streptococcus sanguis* evaluated *in vitro*. Lasers Surg. Med. 18, 253–259.
19. Zanin, I.C., Gonçalves, R.B., Vjunior, A.B., Hope, C.K., and Pratten, J. (2005). Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy: an *in vitro* study. J. Antimicrob. Chemother. 56, 324–330.
20. Bertolini, G., Benedetto, S., Dall'acqua, M., Vazzoler, M., and Jori, G. (1984). Hematoporphyrin-sensitized photoinactivation of *Streptococcus faecalis*. Photochem. Photobiol. 39, 811–816.
21. Wilson, M. (1993). Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. J. Appl. Bacteriol. 7, 5299–5306.
22. Wilson, M., Dobson, J., and Sarkar, S. (1993). Sensitization of periodontopathogenic bacteria to killing by light from a low-power laser. Oral Microbiol. Immunol. 8, 182–187.
23. Wilson, M., Burns, T., Pratten, J., and Pearson, G.J. (1995). Bacteria in supragingival plaque samples can be killed by low-power laser light in the presence of a photosensitizer. J. Appl. Bacteriol. 78, 569–574.
24. Malik, Z., Judith, H., and Nitzan, Y. (1990). New trends in photobiology (invited review) bactericidal effects of photoactivated porphyrins—an alternative approach to antimicrobial drugs. J. Photochem. Photobiol. B. Biol. 5, 281–293.
25. Tamietti, B.F., Machado, A.H., Maftoum-Costa, M., Da Silva, N.S., Tedesco, A.C., and Pacheco-Soares, C. (2007). Analysis of mitochondrial activity related to cell death after PDT with AlPCS(4) . Photomed. Laser Surg. 25, 175–179.
26. Ito, T. (1977). Toluidine blue: the mode of photodynamic action in yeast cells. Photochem. Photobiol. 25, 47–53.
27. Paardekooper, M., Bruijne, A.W., Van Steveninck, J., and Van Den Broek, P.J.A. (1995). Intracellular damage in yeast cells caused by photodynamic treatment with toluidine blue. Photochem. Photobiol. 61, 84–89.
28. Burns, T., Wilson, M., and Pearson, G.J. (1993). Sensitization of cariogenic bacteria to kill by light from helium-neon laser. J. Med. Microbiol. 38, 401–405.
29. Venezio, F.R., Divincenzo, C., Sherman, R., et al. (1985). Bactericidal effects of photoradiation therapy with hematopophyrin derivative. J. Infect. Dis. 151, 166–169.
30. Nitzan, Y., Gutterman, M., Malik, Z.B. and Ehrenberg, B. (1992). Inactivation of gram-negative bacteria by photosensitized porphyrins. Photochem. Photobiol. 55, 89–96.
31. Merchat, M., Bertolini, G., Giacomini, P., Villanueva, A., and Jori, G. (1996) Meso-substituted cationic porphyrins as effi-

cient photosensitizers of gram-positive and gram-negative bacteria. J. Photochem. Photobiol. B. Biol. 32, 153–157.
32. Williams, J.A., Pearson, G.J., Colles, M.J., and Wilson, M. (2004). The photo-activated antibacterial action of toluidine blue O in a collagen matrix and carious dentine. Caries Res. 38, 530–536.

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