Photodynamic inactivation of planktonic cultures and *Streptococcus mutans* biofilms for prevention of white spot lesions during orthodontic treatment: An in vitro investigation

Maria Ângela Lacerda Rangel Esper,[a] Juliana Campos Junqueira,[b] Adjaci Fernandes Uchoa,[c] Eduardo Bresciani,[a] Alessandra Nara de Souza Rastelli,[a] Ricardo Scarparo Navarro,[c] and Sérgio Eduardo de Paiva Gonçalves[a]
São Paulo, Brazil

Introduction: This study evaluated the efficacy of photodynamic inactivation (PDI) with hematoporphyrin IX (H) and modified hematoporphyrin IX (MH) at 10 μmol/L, using a blue light-emitting diode (LED), fluence of 75 J/cm,^{2} over planktonic cultures and biofilm of *Streptococcus mutans* (UA 159). **Methods:** Suspensions containing 107 cells/mL were tested under different experimental conditions: a) H and LED (H+L+), b) MH and LED (MH+L+), c) only LED (P−L+), d) only H (H+L−), e) only MH (MH+L−), and f) control group, no LED or photosensitizer treatment (P−L−). The study also evaluated the effect of PDI on *S mutans* biofilm on metallic or ceramic brackets bonded on specimens of human teeth. The strains were seeded onto *Mitis salivarius*–bacitracin–sacarose agar to determine the number of colony-forming units. **Results:** H and MH under LED irradiation were effective on planktonic cultures ($P < 0.0001$). H and MH (H+L+ and MH+L+) caused a reduction of 3.80 and 6.78 $\log^{10}$ CFU/mL. PDI with the use of H or MH and LED exerted a strong antimicrobial effect over *S mutans* showing 54% and 100% reduction, respectively. PDI on *S mutans* biofilm on metallic and ceramic brackets with the use of H was not effective ($P = 0.0162$, $P = 0.1669$), however, MH caused a significant reduction of 44% and 53% of the cell count on metallic and ceramic brackets, respectively ($P = 0.0020$, $P = 0.004$). **Conclusions:** In vitro planktonic cultures with the use of H or MH and LED exerted significant antimicrobial activity. No effect was observed on *S mutans* biofilm on either bracket type with the use of H, MH showed better results, suggesting a promising use against dental caries and white spot lesions. (Am J Orthod Dentofacial Orthop 2019;155:243-53)

Although orthodontic treatment has advanced with innovative material and techniques, some aspects still need further study, such as pain sensitivity reported by patients after orthodontic movement[1] and preventive methods to avoid white spot lesions and decay during orthodontic treatment with the use of fixed appliances.[2,3]

The placement of removable and/or fixed orthodontic appliances causes modification in the oral ecosystem, increasing the number of cariogenic bacteria.[4,5] It also favors the formation of biofilm and restricts salivary self-cleaning, and oral hygiene becomes more difficult.[6,7] An imbalance occurs between the processes of demineralization and remineralization, increasing the risk of demineralization and decay and

[a]Department of Restorative Dentistry, Institute of Science and Technology, São Paulo State University, São Paulo, Brazil.
[b]Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, São Paulo State University, São Paulo, Brazil.
[c]Department of Biomedical Engineering, Anhembi Morumbi University, São Paulo, Brazil.
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Address correspondence to: Maria Ângela Lacerda Rangel Esper, Department of Restorative Dentistry, Institute of Science and Tecnology, São Paulo State University–UNESP, Avenida Engenheiro Francisco José Longo, 777, Jardim São Dimas, São José dos Campos, SP, CEP 12245-000, Brazil; e-mail, angela_esper@hotmail.com.
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the development of periodontal inflammation, with notable changes in the microbiota. Many clinical studies have shown that patients undergoing fixed orthodontic treatment are more susceptible to periodontal diseases and white spot lesions (WSLs), regardless of the type of bracket used,[8-11] especially if the patient does not cooperate with oral hygiene.[6,12-15]

Demineralization around the brackets is an adverse effect of great clinical relevance, despite modern advances in the prevention of dental caries.[6,16,17] It is well known that WSLs can be seen as early as 4 weeks after the start of treatment with fixed orthodontic appliances,[18,19] especially in the cervical region and around the brackets,[12] if preventive measures are not established[20] and depending on the host conditions (highly acidic saliva, cariogenic diet).[21]

The usual methods to remove the oral microbial biofilm are tooth brushing, use of dental flossing and antiseptics. However, biofilm removal depends on patient compliance, and the use of antiseptics could produce drug-resistant microbes and lead to other side-effects.[22] It is necessary to highlight the importance of diet orientation for the patients during orthodontic treatment to prevent WSLs and tooth decay.[23]

Photodynamic therapy (PDT) has been applied in many areas of medicine and dentistry with effective results.[24,25] Antimicrobial PDT or photodynamic inactivation (PDI) might be an alternative for biofilm control or removal, prevention of WSLs, dental caries, and periodontal diseases[26] with the use of a light source (halogen lamps, laser, or light-emitting diode [LED]) to activate a specific photosensitizer in the presence of oxygen, producing reactive radicals that induce cell death.[27] The use of PDI to treat different oral infections has important advantages over conventional therapeutic treatments. It can promote effective antimicrobial activity without the development of microbial resistance and can be used repeated times without limitation of total dose.[27,28]

There are many photosensitizers that can be used for PDI, and the amount of singlet oxygen generated can change for each photosensitizer or dye. This is called the singlet oxygen quantum yield (QY), and the QY of hematoporphyrin derivatives are high[29] and an advantage compared with other photosensitizers. Hematoporphyrin IX is derived from protoporphyrin and has been used as a photosensitizer for PDT. However, its application is limited owing to its aggregation and low solubility in a physiologic medium. Modifications to the protoporphyrin IX molecule have been made to increase the solubility in a physiologic medium and stimulate its use in PDT. Thus, hydroxyl groups have been inserted in the vinylic positions 3^1, 8^1, 13^3, and 17^3 to produce modified hematoporphyrin IX in both acid and basic media and to potentialize global income.[30,31]

LEDs are commonly used in the dental office for light-curing composite resins and recently for tooth whitening and has many advantages compared with laser, such as low cost and simplicity.[32] The emission spectrum of the blue LED matches the activation spectrum of the photosensitizers used in the present study.[33] There are no papers in the literature describing the effect of PDI with the use of hematoporphyrin IX or modified hematoporphyrin IX and LED on planktonic cultures and biofilms of *Streptococcus mutans* on human teeth specimens bonded with metallic and ceramic brackets.

Therefore, the purpose of the present study was to evaluate the effect of PDI with the use of hematoporphyrin IX or modified hematoporphyrin IX (experimental) photosensitizers irradiated by a blue LED on the viability of planktonic cultures and biofilms of *S mutans* on metallic and ceramic brackets. The null hypothesis was that hematoporphyrin IX and modified hematoporphyrin IX do not decrease the numbers of CFU/mL on planktonic cultures and biofilms of *S mutans*.

MATERIAL AND METHODS

This study was submitted to the Institutional Review Board of São Paulo State University—UNESP (CONEP; CAAE: 44832215.0.0000.0077; IRB: 1.190.860) because of the use of human teeth (third molars) indicated for extraction.

Specimen preparation

Eighty cylinders of 6.0 mm × 2.15 mm (n = 10 for each group) were obtained with the use of a diamond trephine drill from 30 recently extracted human impacted third molar teeth (2 or 3 specimens from each tooth), without any caries or visible defects and kept in 0.1% thymol. After cutting, the cylinders were polished (DP-10; Panambra, São Paulo, Brazil) with the use of silicon-carbide abrasive paper (grit #1200; Extec Corp, Enfield, Conn) for 30 seconds at 300 rpm. After polishing, the specimens were immersed in an ultrasonic bath for 10 minutes (Ultrasonic Cleaner; Odontobras, Ribeirão Preto, Brazil). The final specimens measured 6 mm diameter and 2 mm height as verified by means of a digital caliper (Starrett, São Paulo, Brazil).

Before bonding technique, the buccal surface of each specimen was cleansed and polished by means of water and nonfluoridated pumice mixture with the use of a low-speed handpiece for 6 seconds. Each specimen was etched with the use of 37% phosphoric acid gel (Gel Etch; 3M Unitek, Monrovia, Calif) for 30 seconds,

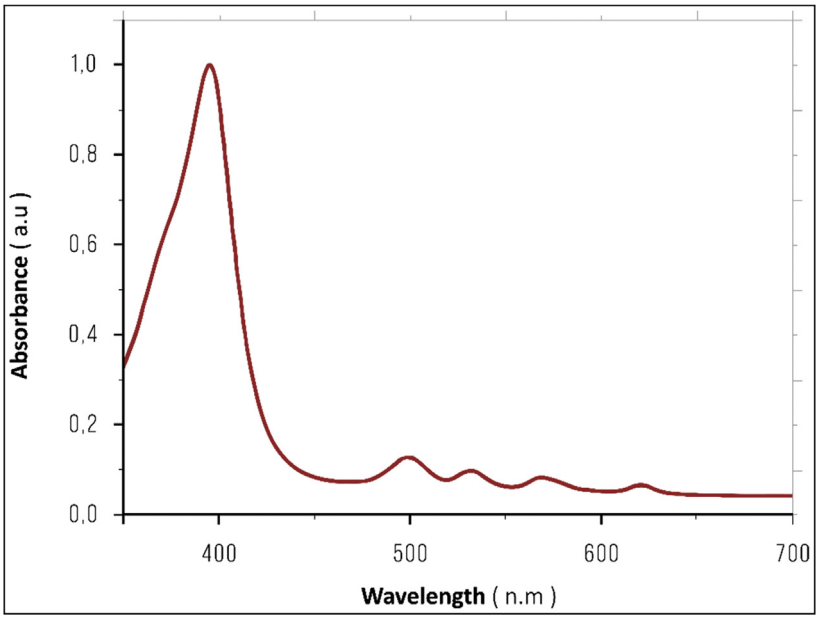

Fig 1. Absorption spectrum (nm) of hematoporphyrin IX and modified hematoporphyrin IX photosensitizers.

rinsed for 10 seconds, and air dried for 5 seconds. A thin uniform layer of sealant (Orthoprimer, ref 85.01.016; Morelli, Sorocaba, Brazil) was brushed on the etched enamel surface and on the metallic and ceramic bracket base. The paste (Orthobond, ref 85.01.015; Morelli) was placed onto the bracket base with the use of a spatula for composite resin. An explorer probe was used to seat the bracket on the enamel surface with a consonant force, and excessive adhesive was removed. The composite resin was light cured with the same visible light-curing unit used for PDI at 1200 mW/cm^2 (Emitter C; Schuster, Santa Maria, Brazil) for 40 seconds (10 s-on the distal, 10 seconds on the mesial, 10 seconds on the incisal, and 10 seconds on the gingival surface of the bracket). In this study, a 0.022-inch inferior incisor metallic (ref 10.30.209; Morelli) and ceramic (ref 10.18.012; Morelli) brackets were bonded on the enamel surface. After bonding, the specimens were kept in deionized water at 37°C. Before the experiments, described below, specimens with bonded brackets and a metallic supports were sterilized by means of autoclaving in Falcon tubes containing deionized water for 30 minutes at 135°C.

Photosensitizers and light source

Hematoporphyrin IX ($C_{36}H_{42}N_4O_6$) and modified hematoporphyrin IX ($C_{50}H_{74}N_8O_2$) dihydrochloride synthesized at the Institute of Chemistry (São Paulo University—USP, São Paulo, Brazil) were used at concentrations of 10 μmol/L for the sensitization of *S mutans*. The photosensitizer solutions were prepared by dissolving the dye in dimethyl sulfoxide (DMSO; Merck) and 0.85% sodium chloride. After filtration through a sterile 0.20-μm Millipore membrane (GVS, Sanford, Me), the photosensitizer solutions were stored in the absence of light. Figure 1 shows the absorption spectrum for hematoporphyrin IX and modified hematoporphyrin IX, and Figure 2 their chemical structures.

The light source used was a wireless blue LED (Emitter C; Schuster) at a wavelength of 420-480 nm, power output of 625 mW, and an illuminated area of 0.5 cm^2. A fluence of 75 J/cm^2 (energy of 37.5 J and irradiation time of 60 s) and a fluence rate of 1250 mW/cm^2 were used. Emission spectrum of the blue LED is shown in Figure 3.

Microorganism and production of the biofilms

The planktonic culture methodology was as described by Costa et al[34] and the biofilm formation as described by Pereira et al.[26] The reference strain (UA 159) of *S mutans* maintained in our laboratory stock collection was used in this study. Standard suspension containing 10^7 cells/mL was prepared. For this purpose, *S mutans* was seeded onto brain heart infusion (BHI) and incubated for 24 hours at 37°C (±1°C) under microaerophilic conditions. After incubation, the microorganisms were cultured in 7.5 mL BHI broth (Himedia) for 24 hours at 37°C (±1°C) under

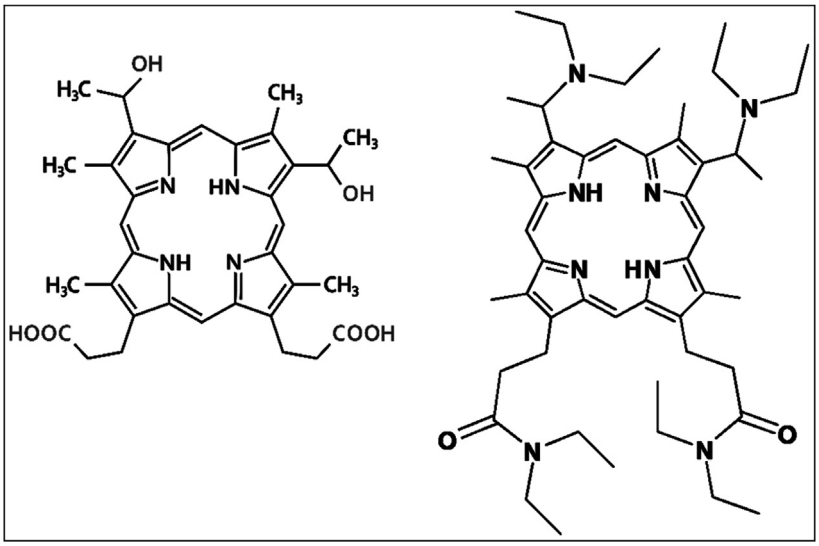

Fig 2. Chemical structure of hematoporphyrin IX (*left*) and modified hematoporphyrin IX (*right*) photosensitizers.

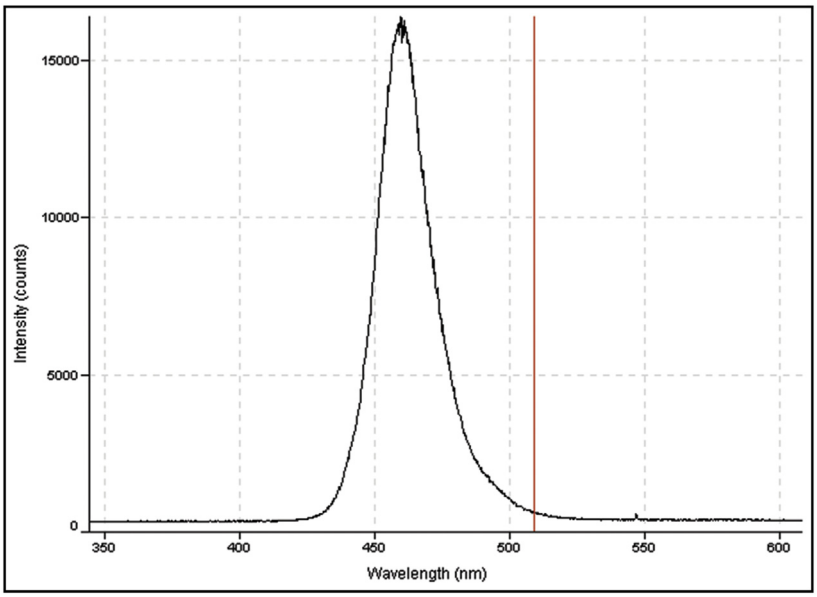

Fig 3. Emission spectrum of the blue LED (Emitter C; Schuster, Santa Maria, Brazil).

microaerophilic conditions. All incubations were carried out at 37°C (±1°C) in 5% CO_2. The bacterial cultures were then centrifuged at 4700*g* for 5 minutes, the supernatant discarded, and the pellet washed with the use of 5 mL sterile saline solution (0.85% sodium chloride). This procedure was repeated, and the pellet was resuspended in 5 mL sterile physiologic solution.

The number of cells in each suspension was measured in a spectrophotometer (B582; Micronal, São Paulo, Brazil) at a wavelength of 398 nm, and an optical density of 0.560 was reached. These parameters were previously established by means of a standard curve with colony-forming units (CFU) versus absorbance.

The biofilms were grown over sterile metallic or ceramic brackets bonded on specimens of human teeth. The specimens were placed in 24-well plates (Costar Corning, New York, NY), suspended in each well by means of a metallic support (Figs 4,

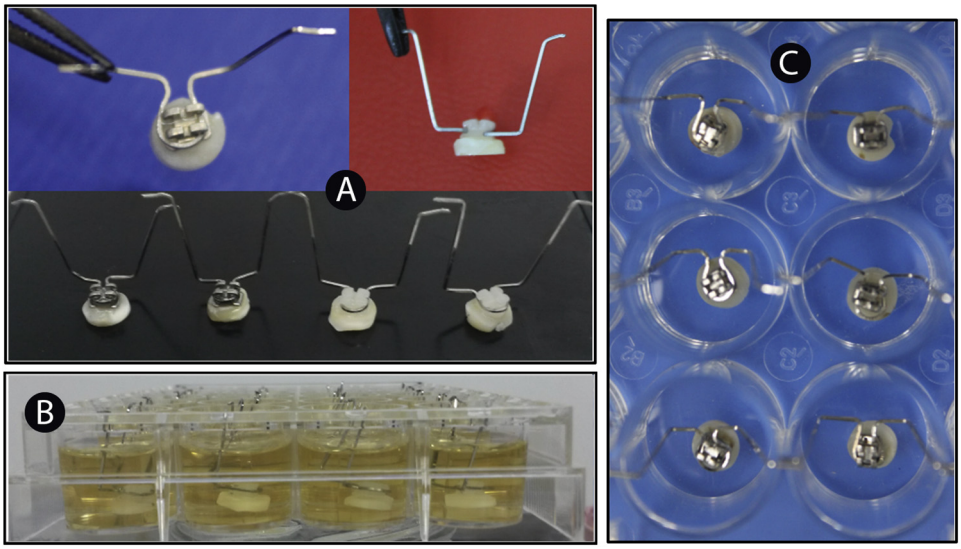

Fig 4. A, Metallic support made with stainless steel orthodontic wire for specimens support. **B,** Specimen placement in a 24-well plate. **C,** Biofilm formation, specimens were suspended, preventing them from touching the well bottoms.

A and *B*) made with stainless steel orthodontic wire (0.016 inch × 0.016 inch, ref 55.02; Morelli), with 2 mL sterile broth and were inoculated with 0.1 mL *S mutans* standard suspensions. The metallic supports were made to suspend the specimens and prevent them from touching the well bottoms, which had more accumulation of biofilm, to simulate the oral cavity (Fig 4, *C*). After 24 hours, the broth was aspirated and refreshed. The plates were incubated for 48 hours.

For each photosensitizer, the assays were submitted to 4 experimental conditions (n = 10 each), as described in the Table, at 2 different times.

In vitro photosensitization of planktonic cultures

According to the experimental conditions described, 0.1 mL bacterial suspension was added to each well of sterile 96-well flat-bottom microtiter plates (Costar Corning). Next, 0.1 mL photosensitizer was added for groups H+L+, MH+L+, H+L−, and MH+L−, whereas 0.1 mL sterile physiologic solution was added for groups P−L+ and P−L−. The plates were shaken for 5 minutes before irradiation in an orbital shaker (Solab, Piracicaba, Brazil). After that, the well content of groups H+L+, MH+L+, P−L+ was irradiated according to the protocol described above. The distance between the light source and the bacterial cells was ~6 mm. Irradiation was performed under aseptic conditions in a laminar flow hood in the dark. The plates were covered with a sterilized matte black screen with an orifice whose diameter corresponded to the size of the well entrance to prevent the spreading of light to neighboring wells. After irradiation, serial dilutions were prepared, and 0.1 mL aliquots of each dilution were seeded in duplicate onto *Mitis salivarius*–bacitracin–sacarose (MSBS) agar plates and incubated for 48 hours at 37°C (±1°C) under microaerophilic conditions. After incubation, the number of CFU/mL was determined.

Table. Experimental groups

Group	*Experimental condition*
H+L+	LED irradiation with hematoporphyrin IX as photosensitizer
H+L−	Hematoporphyrin IX only
MH+L+	LED irradiation with modified hematoporphyrin IX as photosensitizer
MH+L−	Modified hematoporphyrin IX only
P−L+	LED irradiation only
P−L−	No LED irradiation or photosensitizer (control group)

In vitro photosensitization of biofilms

After 48 hours of incubation, the specimens containing the biofilms were aseptically washed twice in sterile 24-well flat-bottom microtiter plates with sterile physiologic solution (0.85% NaCl) to remove loosely bound bacteria. The specimens were then placed, without the metallic supports, in sterile 96-well flat-bottom microtiter plates. According to the experimental conditions described in the Table, 0.15 mL photosensitizer was added for groups H+L+, H+L−, MH+L+, and MH+L− and 0.15 mL physiologic

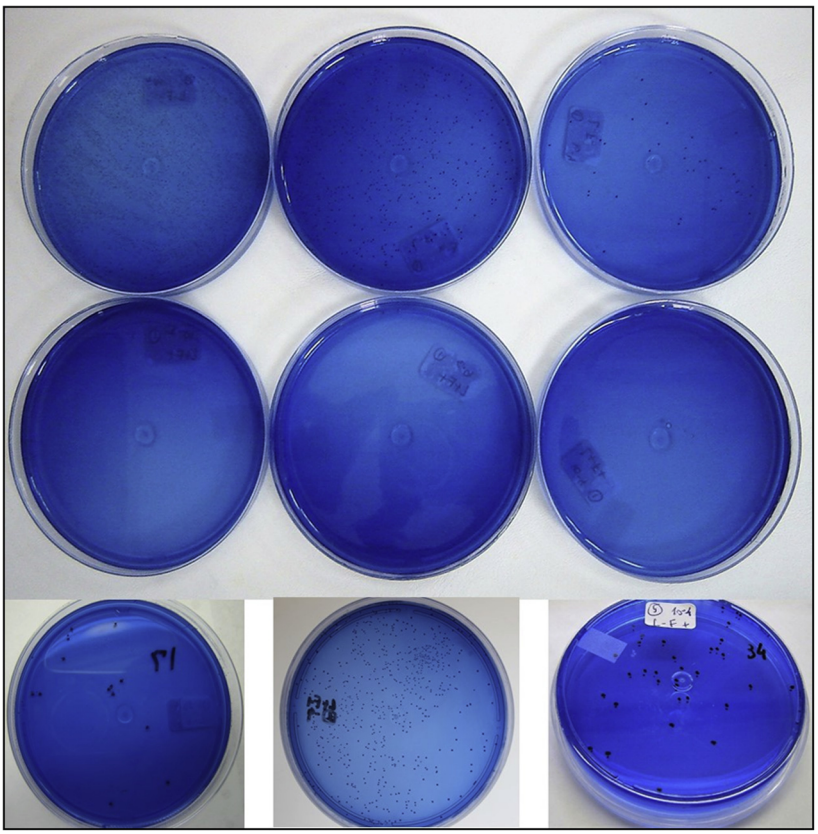

Fig 5. Samples of *Mitis salivarius*–bacitracin–sacarose agar plates and colony-forming units as result of bacteriology.

solution (0.85% NaCl) was added for groups P−L+ and P−L−. The plates were left in the dark and shaken for 5 minutes before irradiation with the use of an orbital shaker (Solab). The biofilms of groups P−L+, H+L+, and MH+L+ were then irradiated according to the protocol described above. The distance between the light source and the bacterial cells and biofilms was ∼6 mm. Irradiation was performed under aseptic conditions in a laminar flow hood in the dark. The plates were covered with a sterile matte black screen with an orifice whose diameter corresponded to the size of the well opening to prevent the spreading of light to neighboring wells. After PDI, the specimens were washed, placed in tubes with 10 mL sterile physiologic solution (0.85% NaCl) and sonicated (Sonoplus HD 2200, 50 W; Bandelin Eletronic, Berlin, Germany) for 30 seconds to disperse the biofilms. Tenfold serial dilutions were carried out, and 0.1 mL aliquots of each dilution were seeded in duplicate onto MSBS agar plates and incubated for 48 hours at 37°C (±1°C) under microaerophilic conditions. After incubation, the number of CFU/mL was determined (Fig 5).

Statistical analyses

The results were log transformed and statistical analyses were carried out with the use of the Graphpad Prism 6.0 program (Graphpad Software, San Diego, Calif). One-way analysis of variance and the Tukey test were used. A P value of <0.05 was considered to indicate a statistically significant difference.

RESULTS

Figures 6-11 present the mean values and standard deviations of the number of CFU/mL ($\log^{10}$) obtained for the different treatment groups studied.

The susceptibility of planktonic cultures of *S mutans* to PDI with the use of hematoporphyrin IX and modified hematoporphyrin IX (Figs 6 and 7) showed a reduction of 3.80 and 6.78 $\log^{10}$ CFU/mL for groups H+L+ and MH+L+, respectively, compared with the control group (P−L−; P <0.0001). Groups H+L−, MH+L−, and P−L+ produced no reduction in the numbers of CFU/mL of *S mutans* compared with the control group (P−L−).

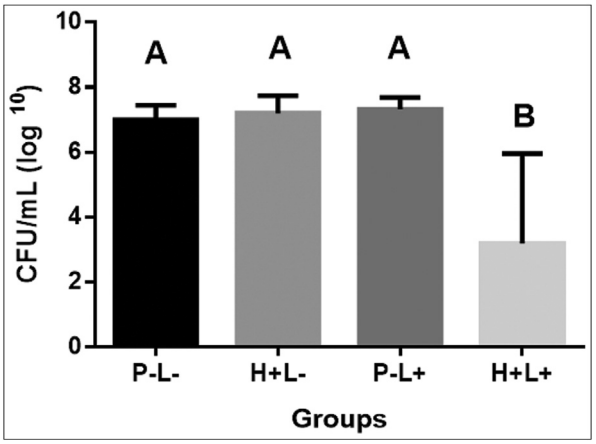

Fig 6. Mean values and standard deviations of the number of CFU/mL (log^{10}) obtained with the use of PDI on planktonic cultures of *S mutans* mediated by hematoporphyrin IX at 10 μmol/L and blue LED (P <0.0001). Different letters indicate statistical differences between groups.

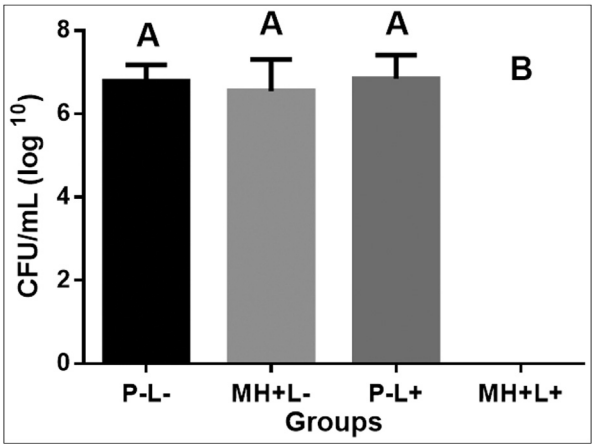

Fig 7. Mean values and standard deviations of the number of CFU/mL (log^{10}) obtained with the use of PDI on planktonic cultures of *S mutans* mediated by modified hematoporphyrin IX at 10 μmol/L and blue LED (P <0.0001). Different letters indicate statistical differences between groups.

The biofilm assays for PDI with the use of hematoporphyrin IX (H+L+, H+L−, and P−L+) produced no reduction in the numbers of CFU/mL of *S mutans* on metallic and ceramic brackets compared with the control group (P−L−; P = 0.0162 and P = 0.1669, respectively), indicating that this photosensitizer was not effective for the species studied (Figs 8 and 9). However, modified hematoporphyrin IX (MH+L+) showed a reduction of 2.43 and 3.60 log^{10} CFU/mL on metallic and ceramic brackets compared with the control group (P−L−; P = 0.0020 and P = 0.0004, respectively), indicating that MH associated with blue LED had a bactericidal effect on *S mutans* biofilm (Figs 10 and 11). Groups MH+L− and P−L+ showed no reduction of *S mutans* compared with the control group (P−L−)

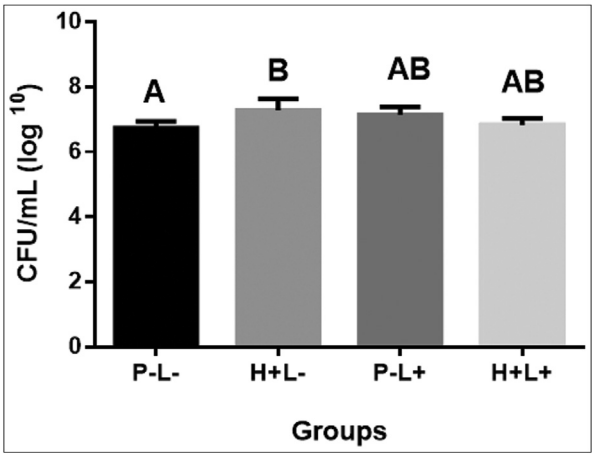

Fig 8. Mean values and standard deviations of the number of CFU/mL (log^{10}) obtained with the use of PDI on *S mutans* biofilms on metallic brackets mediated by hematoporphyrin IX at 10 μmol/L and blue LED (P = 0.0162). Different letters indicate statistical differences between groups.

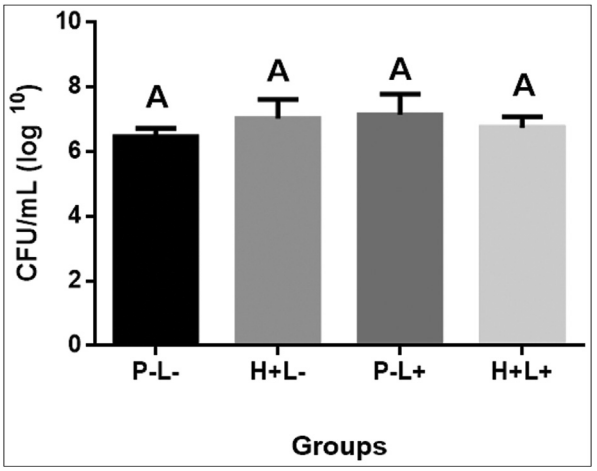

Fig 9. Mean values and standard deviations of the number of CFU/mL (log^{10}) obtained with the use of PDI on *S mutans* biofilms on ceramic brackets mediated by hematoporphyrin IX at 10 μmol/L and blue LED (P = 0.1669). Different letters indicate statistical differences between groups.

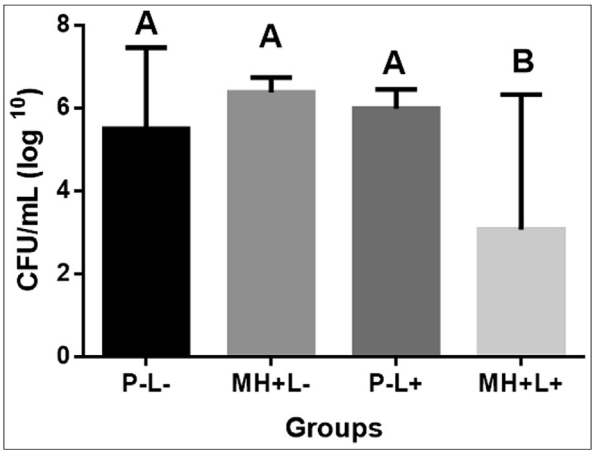

Fig 10. Mean values and standard deviation of the number of CFU/mL (log^{10}) obtained with the use of PDI on *S mutans* biofilms on metallic brackets mediated by modified hematoporphyrin IX at 10 μmol/L and blue LED ($P = 0.0020$). Different letters indicate statistical differences between groups.

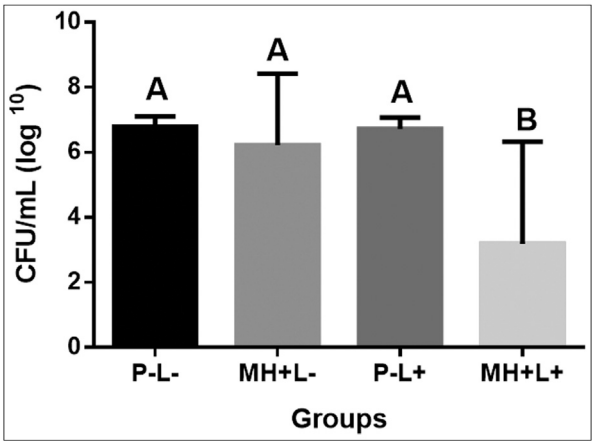

Fig 11. Mean values and standard deviation of the number of CFU/mL (log^{10}) obtained with the use of PDI of *S mutans* biofilms on ceramic brackets mediated by modified hematoporphyrin IX at 10 μmol/L and blue LED ($P = 0.0004$). Different letters indicate statistical differences between groups.

DISCUSSION

Many techniques have been studied for the removal or control of dental biofilm, which is one of the most important etiologic agents for many oral diseases, including dental caries, but with limited success. Therefore, alternate techniques that do not depend on patient compliance are required to prevent WSLs, caries,[22] and periodontal disease in orthodontic patients.[35]

PDI is an alternate method to overcome the microbial resistance of antibiotic drugs and chemical agents. PDI combines an appropriate photosensitizer and light source that is minimally invasive and nontoxic to control the formation of oral biofilm.[22,36] There are some advantages of PDI over conventional antimicrobial agents, such as the killing of the target microorganisms being quick, depending on the parameters used, the lack of resistance by the target organisms, and that the antimicrobial effects can be localized to a specific site, avoiding disruption of normal microflora in other regions.[27,28,37,38]

The success of PDI is based on factors such as type, concentration, preirradiation time of the photosensitizer, physiologic condition of the microorganisms studied, the light source, and the doses used.[39] Therefore, in vitro investigation with the use of a planktonic model is a major step for guiding in vivo protocols and parameters for more detailed future studies when considering illumination aspects (related to the light sources) and concentration (related to the dyes).[40] The in vitro planktonic essays are also essential to determine the efficacy of potential antimicrobial agents. Most methods for evaluating antimicrobial activity of new antimicrobial candidates are based on determining cell viability in microbial suspensions.[41] According to the American Society of Microbiology, a CFU reduction of 3 log^{10} is necessary to use the term "antimicrobial."[42] Tests on planktonic or biofilm cultures may be performed when carrying out microbiologic studies in vitro. In planktonic cultures, microorganisms are free or in small groups and there is an abundant availability of photosensitizer molecules. However, in biofilm communities a more complex organization is observed, forming a 3-dimensional structure with multiple cell layers. It also occurs in the extracellular polysaccharide matrix, with the incorporation of compounds that favor intercellular adhesion, providing an increased resistance to chemical procedures or antimicrobial compounds. Therefore, similar PDI protocols can result in different death rates in experiments on planktonic cultures compared with biofilms.[43]

To establish a future clinical treatment protocol with the use of PDI, there is a need for an evaluation of the efficiency and definition of proper parameters. First, in vitro experiments on planktonic cultures must be carried out. If these experiments are not effective, this treatment technique is likely to be unsuccessful. But even if effectiveness is achieved in cell cultures, it is not necessarily achieved in vivo. However, this sequence of experiments, in vitro and later in vivo, is essential for development in the biomedical area to ensure the safety and efficacy of clinical protocols.[43]

The present study showed an interesting result with hematoporphyrin IX and modified hematoporphyrin IX, killing 54% and 100% (P <0.0001) of *S mutans* in planktonic culture, respectively. In addition, no reduction of *S mutans* biofilms was observed with the use of hematoporphyrin IX on both types of brackets. However, modified hematoporphyrin IX showed a significant inactivation of *S mutans* biofilms, with 44% and 53% survival rate (P = 0.0020 and P = 0.0004), respectively, seen on metallic and ceramic brackets. This is the first study using hematoporphyrin IX and modified hematoporphyrin IX as photosensitizers on planktonic culture and biofilms of *S mutans*.

Hematoporphyrin IX and modified hematoporphyrin IX absorb light at 400 nm, and the light source used in the present study emits light at the range of 420-480 nm. The association between the dyes and light resulted in cellular death, probably due to the generation of reactive oxygen species. The emission spectrum of the blue LED matches the activation spectrum of the photosensitizers used in this study, which is a basic requirement for PDI.[33] Significant differences in PDI with the use of modified hematoporphyrin IX were observed. The results suggested that modified hematoporphyrin IX was more effective than hematoporphyrin IX in the biofilms experiments. The null hypothesis was rejected for both photosensitizers in the first part (planktonic culture) and accepted for hematoporphyrin IX and rejected for modified hematoporphyrin IX in the second part (biofilms).

Hematoporphyrin IX and modified hematoporphyrin IX[44,45] are derivatives of protoporphyrin, which naturally occurs in hemoglobin, cytochrome C, and other biologically relevant molecules. It has been used as a photosensitizer for PDT. However, the application is limited owing to aggregation and low solubility in a physiologic medium. Modifications to the hematoporphyrin IX molecule have been made to increase the solubility of this potential drug in a physiologic medium and to encourage its use in PDT. An important strategy is to explore the reactivity of vinyl groups to functionalize Pp IX with polar groups. To this end hydroxyl groups have been inserted in the vinylic positions 3^1 and 8^1 to produce hematoporphyrin IX. The hematoporphyrin IX, in both acid and basic media, polymerizes to form a mixture of chromophores named Photofrin, which is the most used drug in PDT. Further functionalization of carboxylic groups has also been used for the preparation of several derivatives.[4,5] Because this chemical compound is a hemoglobin derivative, it is well metabolized and it does not have the disadvantage of other compounds that may stain the dental structures, esthetic dental restorations, and even esthetic brackets when used in dentistry.[30,31]

The light source used in this study was based on a blue LED, which presents many advantages.[27,33,46] Dentists could use their light-curing unit, which is based on blue, to perform PDI in their offices. Some studies have shown that the use of LED alone exerts little or no antimicrobial activity.[32,47]

According to Reddy et al (2012),[48] there are 2 basic mechanisms that have been proposed regarding the lethal damage caused to bacteria with the use of PDI. One of them is related to the DNA damage and the other to the damage caused to the cytoplasmic membrane allowing leakage of cellular contents or inactivation of membrane transport systems and enzymes. Breaks in both single- and double-stranded DNA and the disappearance of the plasmid supercoiled fraction have been detected in both gram-positive and gram-negative bacteria. The other potential causes of cell death include the alteration of cytoplasmic membrane proteins, the disturbance of cell wall synthesis, and the appearance of a multilamellar structure near the septum of dividing cells; loss of potassium ions from the cells may also be a possible method of bacterial death.

There is only one longitudinal blind randomized study in the literature of the effect of PDI in patients undergoing orthodontic treatment. The authors used curcumin irradiated by blue LED, compared it with 2% chlorhexidine varnish in adolescent patients during orthodontic treatment, and studied periodontal inflammation.[49] They concluded that PDI represents a promising approach for the prevention of gingivitis in adolescents during fixed orthodontic treatment. However, they reported that further investigations must be carried out to clarify the role of PDI in preventing disease and to encourage studies in larger samples, as well as studies investigating the parameters with the purpose of reducing the light exposure time and optimizing this new treatment technique in patients under high risk of developing various pathologic conditions.

There are no previous studies in the literature on using PDI for the prevention of WSLs in patients undergoing orthodontic treatment. There are important PDI studies in planktonic cultures and *S mutans* biofilms[22,26,34,35,50-54] with interesting inactivation results, but they used other photosensitizers and concentrations and different light sources, doses, and irradiation times. The results of those studies are somewhat adequate regarding microorganisms reduction, but comparison with our data is impaired as a result of the differences in methodology. The previous data were important, however, for proposing

more efficient and more user-friendly protocols. Our study was based on some of those previous works,[26,34] which used an LED wavelength range of 440-460 nm. The same energy, but a different strain of *S mutans* (UA 159), was used in the present study.

Currently, there is a wide variety of orthodontic brackets (metallic, plastic and ceramic conventional, metallic and ceramic self-ligating) that the orthodontist may elect to use with their patients. However, owing to increased demand for orthodontic treatment in adult patients, there is also a high esthetic requirement by the patients and therefore there is a significant increase in the use of ceramic (polycrystalline alumina) and plastic (polycarbonate) brackets.[9] There are several studies on cariogenic bacterial colonization and periodontal changes in the different compositions and bracket designs, as well as in metallic versus elastic ligatures. The authors are unanimous in stating that there is a significant increase of cariogenic microorganisms after the installation of fixed orthodontic appliances, regardless of the type of brackets used. This factor may favor the appearance of WSLs if there is no patient cooperation with oral hygiene and diet orientation.[7-11,19,23] Despite the focus of the present study not being the type of brackets, there was no significant difference in the number of CFU/mL of *S mutans* between the 2 types of brackets, metallic and ceramic, tested. These results are in accordance with the literature.[9,11]

In summary, the results of this study have proved the effectiveness of PDI with the use of modified hematoporphyrin IX on the *S mutans* strain studied, indicating that therapy with the use of this photosensitizer associated with blue LED can be used to prevent WSLs and tooth decay in the near future, because this pathology is usually associated with the presence of *S mutans* biofilm as a potential etiologic agent. New photosensitizer concentrations of hematoporphyrin IX and other doses may be tested to achieve the photodynamic inactivation of the *S mutans* strain UA 159. The synthesis of new and more efficient photosensitizers is an important and interesting approach to improve results in PDI and it is a potential field for new translational and clinical research in the future.

CONCLUSIONS

The present results showed that hematoporphyrin IX and modified hematoporphyrin IX associated with blue LED exerted significant antimicrobial activity in in vitro planktonic cultures. Regarding cultures of *S mutans* in biofilm phase on metallic and ceramic brackets, no effect was observed with the use of hematoporphyrin IX. However, modified hematoporphyrin IX showed better results than hematoporphyrin IX, indicating a promising use against dental caries and WSL prevention.

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