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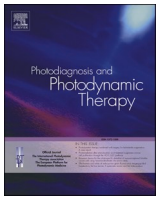

Research Paper

Ultrasonic chamber combined with photodynamic therapy inhibits bacterial growth on dental devices

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ABSTRACT

Background: Photodynamic therapy is a two-stage treatment that combines light energy with a photosensitizer, and enhances the treatment against bacterial infections. In this context, the present study evaluated a newly patented device, called an ultrasonic photodynamic inactivation device (UPID), which performs microbial inactivation using photodynamic therapy for both prosthetic braces (PBs) and prototyped surgical guides (PSGs).
Methods: Photodynamic inactivation was analyzed by contaminating the instruments with bacterial suspensions (3×10^8 CFU/mL) of 100 μM/L methylene blue solution for 20 min, followed by irradiation (0.30 J/cm^2) with red light-emitting diode (660 nm) for 20 min, on three types of microorganisms: *Candida albicans* ATCC 10,231, *Staphylococcus aureus* ATCC 25,923 and *Escherichia coli* ATCC 25,922. The PSGs included a group with irradiation for 30 min (0.45 J/cm^2), and a control group with 0.2 % peracetic acid, evaluated at both 20-minute time points. Microbial inhibition was assessed by counting the number of colony-forming units (CFU), and by the data evaluated using the Shapiro-Wilk, Mann-Whitney-U, and Kruskal-Wallis tests, at a 5 % significance level.
Results: All experimental treatments showed significant reduction in log CFU/mL. The UPID promoted a significant microbial reduction ($p < 0.001$), compared with the positive control. In addition, peracetic acid was more effective than PDT for the PSG ($p < 0.001$). However, after 20 min, both treatments protected the surface material against bacterial growth.
Conclusion: The device proved effective for microbial inhibition of PB and PSG, thus proposing a new technique for the non-toxic disinfection of biomedical devices.

1. Introduction

The main role of photodynamic therapy (PDT) is to form reactive oxygen species by irradiating a light source with a wavelength suitable for a non-toxic photosensitizer (PS), thereby producing microbial inhibition effects [1]. These reactive species, such as singlet oxygen ($^1O_2$), exert strong cytotoxic action on target cells, especially microorganisms [2]. The reagents, formed by mediating the action of the PS, can react with the molecules in their vicinity by electron transfer, to produce reactive species such as hydroxyl radicals or superoxide anions, or by energy transfer to oxygen, to produce $^1O_2$ [3–5]. This element can interact with other molecules through chemical reactions, or transfer its excitation energy to these molecules, and then return to the ground state [6]. Several studies have shown that $^1O_2$ oxidizes biomolecules, including lipids, proteins, amino acids, nucleic acids, carbohydrates, and thiols, through several chemical reactions [6,7].

In this context, microorganisms such as bacteria, fungi, yeasts, and viruses can also undergo inhibitory actions by the reagents [8–10], which are formed by the complementarity of visible light and an adequate PS. This process is known as photodynamic inactivation (PDI) [1,11,12]. The most active PSs used in PDI belong to different groups of compounds, such as halogenated xanthenes (Rose Bengal - RB) and phenothiazines (Toluidine Blue - TB and Methylene Blue - MB) [13]. These PSs belong to the phenothiazine class, long known for playing an

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important role in microbiology, pharmacology, and as a histological dye [12,14,15]. The action of these PSs is well known, because they are interposed in the structure of nucleic acid, resulting from their positive charge (cation) and flat surface area [13]. Thus, MB can be considered a good PS option in PDT for general microorganism inhibition [9,10]. In addition, MB has low toxicity and no side effects, [8,9], and its absorption occurs between 500 and 700 nm, with a peak at approximately 660 nm.

In dentistry, PDT is mainly used as an adjuvant in periodontal, peri-implant and endodontic therapy, and in caries lesion prevention [16, 10,17,18]. Phenothiazine derivatives, such as MB and TB, are the most widely studied PSs in treating oral infections, since they are inexpensive, and the PDT procedure is easily applied in dental clinics.

Periodontal disease is an infectious disease that affects periodontal tissues such as gingiva, cementum, periodontal ligament, and supporting bone [10,19,20]. The progression from healthiness to infectious disease is characterized by an alteration in the subgingival microbiota, with a shift from a gram-positive microbiota to a gram-negative pathogen [21]. The microbiota associated with osseointegrated implants is similar to that of natural teeth. In the case of peri-implantitis, the composition of the peri-implant biofilm is similar to that of the microorganisms found in periodontal lesions [22]. Antiseptics and antibiotics generally have multiple intracellular targets; however, they increase the possibility of developing unwanted bacterial resistance. In this case, they can prevent infection in treatments with prostheses on implants [1, 2,9,10,12–14,23].

In addition, implantology procedures require that keys and prosthetic components be sterilized to minimize the risk of associated peri-implantitis, since a surface free of microorganisms increases the chances of epithelial adhesion in the case of the components, thus reducing the risk of infections associated with prostheses on implants [23]. Nevertheless, although these instruments are metal and therefore subject to sterilization using autoclaves, what is commonly observed in clinical practice is very different. The high cost of prosthetic kits means that most professionals resort to disinfection of the same set of prosthetic braces in consecutive sessions, using 70 % hydrated ethyl alcohol. Thus, PDT application would be a more recommended procedure in these situations. Along the same lines, prototyped surgical guide decontamination is essential, since contamination during implant installation is high, and could compromise the entire process of osseointegration [17, 18]. Currently, the recommended method is either 0.2 % peracetic acid or 2 % chlorhexidine digluconate, both for a 30-min immersion period, since they are thermosensitive materials. However, there are drawbacks, such as cytotoxicity and bacterial resistance, unlike the PDT procedure.

Therefore, this study aimed to evaluate the antimicrobial effect of PDT, using a newly patented device called an ultrasonic photodynamic inactivation device on contaminated prosthetic keys and prototyped surgical guides. The null hypothesis tested is that this new device does not inhibit bacterial growth on the surface of dental materials.

2. Materials and methods

2.1. Ultrasonic photodynamic inactivation device (UPID)

The UPID (MU-BR 20.2018.009356–3) was constructed using a perforated stainless steel metal basket with a polypropylene lid. Irradiation was improved by covering the inside of the lid with a thermal blanket (2 mm) of expanded polyethylene with aluminized polyester (Etaflon, São Paulo) (Fig. 1). In addition, 28 waterproof red light emission diode (LED, wavelength 660 nm) plates were used, and contained three radiators on each 2 W plate (Rohs, China). A 12 V source with 2.5 mA continuous current (Delta Electronics, China) was used to maintain the system. The distribution of the spectral irradiances was measured with a lux meter (THAL-300 Instrutherm, São Paulo), whose parameters were calculated for the 6 internal faces of the device. The UPID was built so that it could be soaked in any ultrasonic bath – this study used model Dabi Atlante 3 L (Ribeirão Preto, São Paulo).

Fig. 1. Ultrasonic photodynamic inactivation device.

2.2. PS

An aqueous solution of MB (Synth, São Paulo, Brazil) was prepared at the biology laboratory of the State University of Paraná, using distilled water and 70 % alcohol (v/v) in 100 μmol/L [24]. Fig. 2 shows the spectrophotometer with the peak absorption of the PS.

2.3. Microorganisms

C. Albicans ATCC 10,231 yeast, *S. aureus* ATCC 25,923, and *Escherichia coli* ATCC 25,922 bacteria were supplied by the São Leopoldo Mandic Institute microbiology laboratory. The microorganisms were added to 50 μl sterile BHI broth. Growth was carried out under

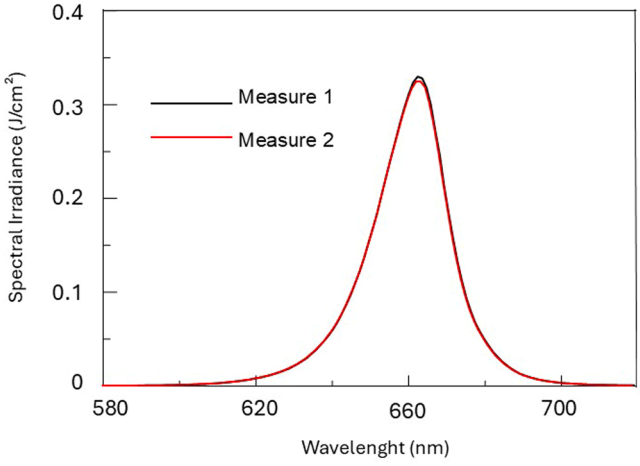

Fig. 2. Spectrophotometer showing the peak absorption of the photosensitizer.

microaerobic conditions at 36 °C for 24 h, until obtained at a stationary stage. Subsequently, the bacterial solutions were diluted using a McFarland scale equivalent to 3×10^8 CFU/mL for the PDI procedure on the prosthetic keys and prototyped surgical guides.

2.4. Prosthetic braces (PBs) and experimental groups

Seventy-two PBs (Neodent, Curitiba, Brazil) were previously sterilized in an autoclave at the São Leopoldo Mandic Institute microbiology laboratory (Fig. 3). The instruments were classified into 4 groups (n = 18/group): G1 – negative control (not contaminated), G2 - *C. albicans*, G3 - *S. aureus*, and G4 - *E. coli*. Before submitting the instruments to PDI, 6 instruments from each group were randomly chosen to compose the positive control group (just contaminated). The remaining 12 instruments in each group underwent PDI, and samples of the surface (active part of the prosthetic key) were collected at the end of the laboratory processes, and spread in a Petri dish (10 μL) containing brain heart infusion (BHI) for observation of colony forming units (CFU). Thus, the number of colonies of each instrument was counted, and compared with the colonies of the respective positive control group.

2.5. Prototyped surgical guides (PSGs) and experimental groups

A total of 108 prototyped thermoset polymer surgical guides were used, made by three-dimensional printing based on a previous virtual model (Smart Solutions, Rio de Janeiro, Brazil) (Fig. 4). Eighteen non-contaminated PSGs were chosen randomly as the negative control. The remaining 90 guides were distributed into 3 groups (n = 30/group): G1 - *C. albicans*, G2 - *S. aureus,* and G3 - *E. coli*. Before subjecting the devices to PDI or 0.2 % peracetic acid (Rioquímica, São José do Rio Preto, Brazil), 6 instruments from each group were randomly chosen to compose the positive control (only contaminated). The remaining 24 guides in each group were divided into four subgroups (n = 6/group), according to the type of treatment: PDI (20 min), PDI (30 min), 0.2 % peracetic acid (20 min), or 0.2 % peracetic acid (30 min), immersed in 200 mL polypropylene bags. At the end of the laboratory processes, specimens from the surface (metal washer located in the central part) were collected and spread in a Petri dish for CFU observation. Then, the number of colonies of each PSG was counted and compared with the number of colonies of the respective positive control group.

2.6. PDT and CFU count

The effect of PDT on microorganisms was evaluated using *C. Albicans* ATCC 10,231, *S. aureus* ATCC 25,923, and *E. coli* ATCC 25,922 at the stationary stage, diluted in phosphate-buffered saline (PBS) (McFarland Scale-3 $\times$ 10^8 CFU/mL) at 600 nm Spectrophotometer SP220, Biospectro, Brazil) with subsequent dilution at 1:10. PBs and PSGs were placed in a glass container (10 $\times$ 20 cm) containing 300 mL for each microbial strain, respectively diluted and stored for 25 min at room temperature. After the initial growth period, a PDT procedure was performed to inhibit bacterial growth. The instruments and devices were placed in separate polypropylene bags containing 200 mL of MB (100 μmol/L) for 20 min. Subsequently, the UPID was used for PDI treatment for 20 min at 0.30 J/cm^2, 75 mW [24,25]. In addition, PDI of the PSGs was performed for 30 min on the three types of microorganisms. The instruments and devices were removed and rubbed in sterile PBS, followed by a 10-min wait. Next, the surface of a 50 mm diameter Petri dish containing the BHI was scored. The dishes were placed in a microaerophilic (Tecnal TE-399) incubator with 5 % oxygen, 10 % carbon dioxide, and 80 % nitrogen, and then incubated for 24 h at 37 °C, until growth was obtained at the stationary stage. The results were analyzed to determine bacterial inhibition according to the growth or non-growth of bacterial colonies. The CFU count was performed with a Phoenix CP608 loupe (Phoenix Industry and Commerce of Scientific Equipment). All the specimens in a contamination-free area were analyzed (laminar flow and Bunsen burner) during culture preparation, irradiation and inhibition measurement, and all the materials involved were previously autoclaved.

Fig. 3. Prosthetic braces.

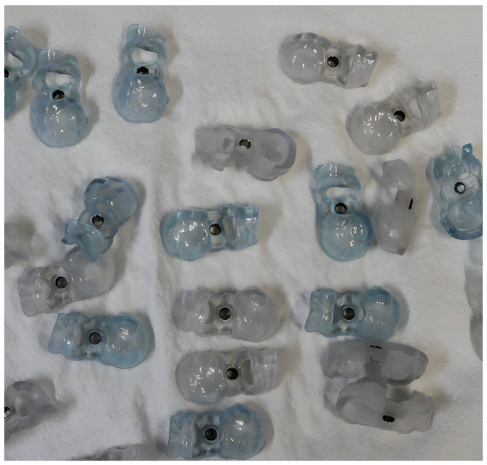

Fig. 4. Prototyped surgical guides.

2.7. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) - version 25. Data normality was tested using the Shapiro-Wilk test. Since the CFU variable did not present a normal distribution for all microorganisms and forms of treatment (Shapiro-Wilk Test: $p < 0.001$ for PB and $p < 0.05$ for PSG), the Mann-Whitney-U test was used to compare the mean CFU with the positive control, in the PB group. The Mann-Whitney test was also used to compare mean colony growth in the PDT- and peracetic acid-treated experiments with the positive control, in the PSG group.

The non-parametric Kruskal-Wallis test was used to analyze the results of the differences among the three PB groups after PDT, and the differences among the PSGs treated with PDT and peracetic acid, according to the different time points and the controls. A significance level of 5 % was used ($p < 0.05$).

3. Results

3.1. PB

The negative control values were zero in the three groups. In the positive control, *C. albicans* was 306.16 ± 11.25 CFU, *S. aureus* was 1325.50 ± 31.69 CFU, and *E. coli* was 637.50 ± 26.42 CFU. After PDT, the mean CFU value was significantly lower ($p < 0.001$) (Table 1).

3.2. PSG

Statistical differences were detected between the forms of microbial control and the positive control ($p < 0.05$). Hence, PDT and peracetic acid were effective against the tested microorganisms. Statistical differences were found between both forms of the tested microbial control ($p < 0.001$), with peracetic acid being the most effective. On the other hand, no statistical differences were detected between the 20- min and 30-min time points ($p > 0.05$). Therefore, 20 min is enough for microbial control, in comparison with the positive control using PDT, and with peracetic acid. The negative control CFU values were zero in the three groups. As for the positive control values, *C. albicans* was 23,474.83 ± 2553.25 CFU, *S. aureus* was 60,992.16 ± 4328.47 CFU and *E. coli* was 65,747.50 ± 8035.84 CFU. After PDT (20 min and 30 min), the mean CFU value was significantly lower ($p < 0.001$) (Table 2).

4. Discussion

The results obtained in this study demonstrated that the UPID and the MB concentration used (100 μmol/L) were effective in decontaminating PB and PSG surfaces, using PDT against yeasts, gram-positive and gram-negative cultures. All the microorganisms analyzed in this study design are found in the oral microbiota, [19,20] and frequently associated with peri-implant diseases [26]. Osseointegrated implants are one of the most commonly used treatment options to replace missing teeth. The increase in implant treatments represents a constant challenge to dentists to avoid failures caused by the loss of soft and bone tissues, resulting from local bacterial infections (peri-implantitis) [27].

These infections can be observed both during the surgical stage of the treatment, when implants are installed with prototyped surgical guides, and during the prosthetic stage, when components and devices are used for patient rehabilitation. Although PSGs are single-use devices and not subject to cross-contamination, their surface may have pathogenic microorganisms, which would be minimized by applying PDT. Regarding PBs, the proposed method can improve contamination by offering an effective and safe alternative to the means of disinfection normally used in clinical practice, such as 70 % hydrated ethyl alcohol, thus avoiding cross-infection.

Loss of implants has been the topic of studies in recent years, and cross-sectional studies in patients treated with implants have shown that peri-implant mucositis occurred in 80 % of the patients, and 50 % of the implant sites. Peri-implantitis was identified in 28 %–56 % of the patients, and in 12 %–43 % of the implant sites [26,28]. Hence, the treatment of infections associated with peri-implantitis consists of controlling the bacterial biofilm, and using antiseptic and chemical agents. However, the use of these agents for a long period can cause bacterial resistance.

In fact, dental caries is considered a multifactorial disease characterized by the localized and progressive destruction of tooth structure, a process that results in the colonization of the enamel surface by various microorganisms. Contamination can occur through contact with contaminated surfaces and instruments, such as orthodontic tools used in the oral cavity, which are classified as semi-critical items. Instruments placed on trays, side tables, or counters near the patient may become contaminated after use, even when not in direct contact. These instruments can become contaminated through the deposition of aerosols composed of blood, saliva, tissue, or other organic fluids. In light of this, our research group decided to investigate additional bacterial species. The most well-known bacteria that inhabit the skin's permanent microbiota are staphylococci, particularly *Staphylococcus aureus*. Although these bacteria are commensals of human skin, certain strains can effectively adhere to solid surfaces and form biofilms, which are a

Table 2
Characterization and comparison of CFU values for the prototyped surgical guides.

Group	Negative control	Positive control	After PDT	*Mann-Whitney-U-Test*[(2)]
C. albicans	0	Average: 306.16 SD: 11.25	Average: 0.58 SD: 0.99	$p < 0.001$[(2)]
S. aureus	0	Average: 1325.50 SD: 31.69	Average: 0.58 SD: 0.79	$p < 0.001$[(2)]
E. coli	0	Average: 637.50 SD: 26.42	Average: 0.33 SD: 0.65	$p < 0.001$[(2)]
Kruskal-Wallis test [(1)] $p > 0.05$				

[(1)] Kruskal-Wallis test significance level for the comparison between the 3 groups.
[(2)] Significance level of the Mann-Whitney-U-Test for comparison with the positive control.
Negative control ($n = 10$); Positive control ($n = 6$), After Photodynamic Therapy (PDT, $n = 12$).

Table 1
Characterization and comparison of CFU values of prosthetic braces.

Group	Negative control	Positive control	After PDT (20 min)	After PDT (30 min)	Teste *Mann-Whitney*[(2)]	After peracetic acid (20 min)	After peracetic acid (30 min)	Teste *Mann-Whitney*[(2)]
C. albicans	0	Average: 23,474.83 SD: 2553.25	Average: 276.50 SD: 62.33 $p < 0.001$[(1)]	Average: 225.17 SD: 33.18 $p < 0.001$[(1)]	$p > 0.05$	Average: 0 SD: 0 $p < 0.001$[(1)]	Average: 0 SD: 0 $p < 0.001$[(1)]	$p > 0.05$
S. aureus	0	Average: 60,992.16 SD: 4328.47	Average: 604,17 SD: 96,57 $p < 0.001$[(1)]	Average: 272.83 SD: 73.42 $p < 0.001$[(1)]	$p > 0.05$	Average: 0 SD: 0 $p < 0.001$[(1)]	Average: 0 SD: 0 $p < 0.001$[(1)]	$p > 0.05$
E. coli	0	Average: 65,747.5 SD: 8035.84	Average: 255.50 SD: 34.73 $p < 0.001$[(1)]	Average: 171.0 SD: 92.60 $p < 0.001$[(1)]	$p > 0.05$	Average: 0 SD: 0 $p < 0.001$[(1)]	Average: 0 SD: 0 $p < 0.001$[(1)]	$p > 0.05$
	Kruskal-Wallis test $p < 0.001$[(3)]					Kruskal-Wallis test $p < 0.001$[(3)]		

[(1)] Significance level of the Mann-Whitney-U-Test for comparison between the treatments (PDT and peracetic acid) and positive control.
[(2)] Significance level of the Mann-Whitney-U-Test for comparison between the treatments time (20 min and 30 min).
[(3)] Kruskal-Wallis test significance level for comparison between the treatments time (20 min and 30 min) and positive control.
Negative control ($n = 6$); Positive control ($n = 6$), After 20 min PDT ($n = 6$), After 30 min PDT ($n = 6$), After 20 min peracetic acid ($n = 6$), After 30 min peracetic acid ($n = 6$).

leading cause of infections when they come into contact with an impaired skin barrier. As well, *Candida albicans* and *E. coli* play a central role in biofilm formation due to their ability to strongly adhere to dental materials.

In this study, significant results were obtained because *C. albicans* and other yeasts showed greater resistance to antimicrobial action when treated with PDT, compared with gram-positive bacteria. This can be attributed to the presence of a membrane nucleus in the yeast structure, and also to greater cell size and a smaller number of $^1O_2$ targets [29]. Another interesting point related to this yeast is that the indiscriminate use of antifungals leads to *Candida* sp. resistance, thus requiring new treatment alternatives for oral candidiasis. The application of PDT has been investigated for inactivation of pathogenic microorganisms in the human host [17,29,30]. Gram-negative microorganisms also indicate resistance to PDT caused by low MB penetration due to the outer membrane of these bacteria [31].

PDI action has already been proven in a new patented device [32]. In this study, the box containing the light-emitting diode proved effective in reducing or disinfecting microbial action on the solid metal surfaces of dental devices using PDT. To the best of our knowledge, this was the first time that the UPID was tested in conjunction with PDT on PBs and PSGs to evaluate bacterial growth (*C. albicans, S. aureus* and *E. coli*) on metal surfaces. Our positive results corroborate those of previous in vitro studies [30,31,33,34]. Oral microbiota is composed of >500 types of microorganisms, although *C. albicans, S. aureus,* and *E. coli* are the main etiological agents of oral pathologies [19]. The presence of pathogenic microorganisms in biofilms can lead to pathological processes such as dental caries, periodontal disease, and peri-implantitis. Additionally, one of the main problems in dentistry is cross-contamination caused mainly by the patient's oral fluids [19,20]. Thus, the clinical dental environment is a pathway that exposes professionals and their patients to biological risks [35].

It is important to highlight that, based on our protocol, effective decontamination has been demonstrated by reducing the number of live bacterial cells on the metal surface of the keys, and the polymeric surface of the guides. The potential clinical benefit is the reduction of infections related to the various stages of implant dentistry treatments. Regarding the pigmentation caused by using MB as a non-toxic PS, pigmentation does not cause any significant clinical impact on the surface, because PBs and PSGs are discarded after implant placement surgery. However, to apply the UPID in clinical settings, several factors must be considered: a) clinical effectiveness: the device demonstrated significant microbial reduction, but additional studies may be needed to assess its efficacy under different clinical conditions (e.g., mature biofilm, saliva, presence of organic matter);b) patient safety: methylene blue and red light are generally safe, but it is essential to ensure no adverse effects on oral tissues; c) application time: in dental practices, 20 min may be feasible for disinfecting devices before procedures, though it may be too long for direct intraoral applications. Alternative protocols with higher intensities and shorter durations should be explored; d) comparison with conventional methods: the study indicated that peracetic acid was more effective for PSGs. Thus, PDT may be particularly useful when chemical-free disinfection is required, reducing potential toxicity and material degradation.

Regarding PB, the Kruskal-Wallis test results ($p < 0.001$) for both PDT and peracetic acid treatments across all microorganisms confirm that the treatments significantly differ from the negative control and have a profound impact on microbial reduction. Both PDT and peracetic acid demonstrate strong antimicrobial efficacy across *C. albicans, S. aureus*, and *E. coli*, with peracetic acid achieving complete eradication in all cases and PDT showing a dose-dependent reduction in microbial load. While PDT is highly effective, particularly in the case of shorter treatment durations, peracetic acid presents an equally potent alternative, with the advantage of complete elimination of the microorganisms within the tested exposure times. Future studies should further investigate the mechanisms behind the differential effects of these treatments and explore their potential clinical applications, including their effectiveness in biofilm disruption and in vivo models.

Furthermore, since PSGs are thermosensitive materials, they are duly indicated for PDT instead of 0.2 % peracetic acid, because of the corrosive potential of the acid on dental instruments and tissue cytotoxicity of the oral cavity, drawbacks not observed in PDT [36]. The time factor is the same for both methods – acid immersion and light irradiation. The Kruskal-Wallis test, used to compare the results among the three groups, found no significant differences ($p > 0.05$) between the species studied after PDT treatment. This suggests that PDT had a similar effect on the three bacterial/fungal species analyzed, reinforcing the idea that PDT can be an effective and broad strategy for reducing various pathogenic microflora. In summary, the data indicate that PD) was effective in reducing *C. albicans, S. aureus*, and *E. coli* compared to the positive controls, suggesting that PDT could be a promising strategy for controlling and reducing microbial load of various pathogens in clinical settings, particularly in the oral cavity.

An additional point to mention is that the absorption peak can change when a PS is combined with alcohol, due to changes in solvent polarity, molecular aggregation, and hydrogen bonding interactions. The direction and magnitude of the shift depend on the specific PS and alcohol used. However, the present study demonstrated a significant microbial reduction ($p < 0.001$), indicating that PDT was effective. If there had been a major shift in the absorption peak, a reduction in PDT effectiveness would have been expected, which was not observed. Since microbial inactivation was successful, any changes in PS absorption were minimal and did not compromise the study's results.

While the study demonstrated significant microbial inactivation with the applied energy density of 0.3 J/cm^2, it is important to note that the minimum power is also a critical factor in the success rate of PDT. The energy density alone does not fully capture the optimal parameters required for effective PDT. Future studies should explore the effects of varying power settings in combination with different light delivery protocols to further optimize treatment outcomes. Additionally, exploring a broader range of energy densities and exposure times could provide valuable insights into achieving the most efficient and effective PDT regimen for microbial inactivation.

Also, peracetic acid was used as a positive control to compare the efficacy of PDT, representing as standard antimicrobial. Although PDT showed significant results in reducing CFU, peracetic acid was more effective. However, PDT still proved to be a viable alternative, especially in situations where the use of more aggressive chemicals is not desirable.

Based on the results presented herein, it can be concluded that the UPID was effective in inhibiting microbial activity by using PDT against *C. albicans*, S. aureus, and *E. coli*, all associated with oral diseases and infections, including microorganisms directly related to peri-implantitis. Moreover, future studies are needed to analyze the application of the present method to other instruments or devices in clinical practice.

5. Conclusions

The results presented in this study indicated that the ultrasonic photodynamic inactivation device was effective for microbial control. This new device may be an alternative for the disinfection of biomedical tools, such as noncritical instruments.

Declarations

Funding

The authors declare no funding.

CRediT authorship contribution statement

Jeter Bochnia Ribeiro: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Augusto**

Alberto Foggiato: Resources, Methodology, Investigation. **Douglas Silva Fernandes:** Software, Methodology, Investigation. **João Victor Frazão Câmara:** Writing – review & editing, Investigation, Formal analysis. **Amanda de Oliveira Pinto Ribeiro:** Methodology, Investigation. **Fabiana Mantovani Gomes França:** Writing – review & editing, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

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References

[1] F. Gad, T. Zahra, T. Hasan, M.R. Hamblin, Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria, Antimicrob. Agents Chemother. 48 (6) (2004) 2173–2178, https://doi.org/10.1128/AAC.48.6.2173-2178.2004.

[2] S.R.D. Gamelas, C. Vieira, M. Bartolomeu, M.A.F. Faustino, J.P.C. Tomé, A. C. Tomé, A. Almeida, L.M.O. Lourenço, Photodynamic inactivation of pathogenic gram-negative and gram-positive bacteria mediated by Si(IV) phthalocyanines bearing axial ammonium units, J. Photochem. Photobiol. B 233 (2022) 112502, https://doi.org/10.1016/j.jphotobiol.2022.112502.

[3] K.C. Sadanala, P.K. Chaturvedi, Y.M. Seo, J.M. Kim, Y.S. Jo, Y.K. Lee, W.S. Ahn, Sono-photodynamic combination therapy: a review on sensitizers, Anticancer Res. 34 (9) (2014) 4657–4664.

[4] I.S. Turan, D. Yildiz, A. Turksoy, G. Gunaydin, E.U. Akkaya, A bifunctional photosensitizer for enhanced fractional photodynamic therapy: singlet oxygen generation in the presence and absence of light, Angew. Chem. Int. Ed Engl. 55 (8) (2016) 2875–2878, https://doi.org/10.1002/anie.201511345.

[5] R. Abdurahman, C.X. Yang, X.P. Yan, Conjugation of a photosensitizer to near infrared light renewable persistent luminescence nanoparticles for photodynamic therapy, Chem. Commun. (Camb.) 52 (90) (2016) 13303–13306, https://doi.org/10.1039/c6cc07616e.

[6] C.L. Hawkins, M.J. Davies, Detection, identification, and quantification of oxidative protein modifications, J. Biol. Chem. 294 (51) (2019) 19683–19708, https://doi.org/10.1074/jbc.REV119.006217.

[7] M.J. Davies, Singlet oxygen-mediated damage to proteins and its consequences, Biochem. Biophys. Res. Commun. 305 (3) (2003) 761–770, https://doi.org/10.1016/s0006-291x(03)00817-9.

[8] X. Shi, C.Y. Zhang, J. Gao, Z. Wang, Recent advances in photodynamic therapy for cancer and infectious diseases, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 11 (5) (2019) e1560, https://doi.org/10.1002/wnan.1560.

[9] A.S. Garcez, S.C. Núñez, Azambuja N Jr, et al., Effects of photodynamic therapy on gram-positive and gram-negative bacterial biofilms by bioluminescence imaging and scanning electron microscopic analysis, Photomed. Laser Surg. 31 (11) (2013) 519–525, https://doi.org/10.1089/pho.2012.3341.

[10] C.R.L. Leal, L.H. Alvarenga, T. Oliveira-Silva, et al., Antimicrobial photodynamic therapy on Streptococcus mutans is altered by glucose in the presence of methylene blue and red LED, Photodiagnosis Photodyn. Ther 19 (2017) 1–4, https://doi.org/10.1016/j.pdpdt.2017.04.004.

[11] G.B. Rodrigues, G.T.P. Brancini, M.R. Pinto, et al., Photodynamic inactivation of Candida albicans and Candida tropicalis with aluminum phthalocyanine chloride nanoemulsion, Fungal. Biol. 124 (5) (2020) 297–303, https://doi.org/10.1016/j.funbio.2019.08.004.

[12] M. Štefánek, L. Černáková, J. Dekkerová, H. Bujdáková, Photodynamic inactivation effectively eradicates Candida auris biofilm despite its interference with the upregulation of CDR1 and MDR1 efflux genes, J. Fungi. (Basel) 8 (11) (2022) 1137, https://doi.org/10.3390/jof8111137.

[13] G. Plotino, N.M. Grande, M. Mercade, Photodynamic therapy in endodontics, Int. Endod. J. 52 (6) (2019) 760–774, https://doi.org/10.1111/iej.13057.

[14] A. Späth, C. Leibl, F. Cieplik, et al., Improving photodynamic inactivation of bacteria in dentistry: highly effective and fast killing of oral key pathogens with novel tooth-colored type-II photosensitizers, J. Med. Chem. 57 (12) (2014) 5157–5168, https://doi.org/10.1021/jm4019492.

[15] F. Cieplik, L. Tabenski, W. Buchalla, T. Maisch, Antimicrobial photodynamic therapy for inactivation of biofilms formed by oral key pathogens, Front. Microbiol. 5 (2014) 405, https://doi.org/10.3389/fmicb.2014.00405. Published 2014 Aug 12.

[16] T.S. Mang, L. Mikulski, R.E Hall, Photodynamic inactivation of normal and antifungal resistant Candida species, Photodiagnosis Photodyn. Ther. 7 (2) (2010) 98–105, https://doi.org/10.1016/j.pdpdt.2010.03.001.

[17] M.A. Melo, J.P. Rolim, V.F. Passos, et al., Photodynamic antimicrobial chemotherapy and ultraconservative caries removal linked for management of deep caries lesions, Photodiagnosis Photodyn. Ther. 12 (4) (2015) 581–586, https://doi.org/10.1016/j.pdpdt.2015.09.005.

[18] M. Nie, D.M. Deng, Y. Wu, et al., Photodynamic inactivation mediated by methylene blue or chlorin e6 against Streptococcus mutans biofilm, Photodiagnosis Photodyn. Ther. 31 (2020) 101817, https://doi.org/10.1016/j.pdpdt.2020.101817.

[19] P.C. Peng, C.M. Hsieh, C.P. Chen, T. Tsai, C.T. Chen, Assessment of photodynamic inactivation against periodontal bacteria mediated by a Chitosan hydrogel in a 3D gingival model, Int. J. Mol. Sci. 17 (11) (2016) 1821, https://doi.org/10.3390/ijms17111821. Published 2016 Nov 1.

[20] D. Wang, H. Pan, Y. Yan, F. Zhang, Rose bengal-mediated photodynamic inactivation against periodontopathogens in vitro, Photodiagnosis Photodyn. Ther. 34 (2021) 102250, https://doi.org/10.1016/j.pdpdt.2021.102250.

[21] J.V. Frazão Câmara, T.T. Araujo, D.A.C. Mendez, N.D.G. da Silva, F.F. de Medeiros, L.A. Santos, T. de Souza Carvalho, F.N. Reis, T. Martini, S.M. Moraes, P.Y. T. Shibao, S. Groisman, A.C. Magalhães, F. Henrique-Silva, M.A.R Buzalaf, Effect of a sugarcane cystatin on the profile and viability of microcosm biofilm and on dentin demineralization, Arch. Microbiol. 203 (7) (2021) 4133–4139, https://doi.org/10.1007/s00203-021-02403-6.

[22] C.A. Pereira, A.C. Costa, C.M. Carreira, J.C. Junqueira, A.O. Jorge, Photodynamic inactivation of Streptococcus mutans and Streptococcus sanguinis biofilms in vitro, Lasers Med. Sci. 28 (3) (2013) 859–864, https://doi.org/10.1007/s10103-012-1175-3.

[23] B.B. Freire, V.A. Wanderley, J.V.F. Câmara, L.A. Santos, C.R. Ferrari, T.T. Araujo, I. Chilvarquer, Effect of a metal artifact reduction algorithm on dehiscence and fenestration detection around zirconia implants with cone beam computed tomography, Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. 138 (2) (2024) 316–323, https://doi.org/10.1016/j.oooo.2024.02.023.

[24] M.L. Zoccolillo, S.C. Rogers, T.S. Mang, Antimicrobial photodynamic therapy of S. mutans biofilms attached to relevant dental materials, Lasers Surg. Med. 48 (10) (2016) 995–1005, https://doi.org/10.1002/lsm.22534.

[25] D.F. Silva, J.L. Toledo Neto, M.F. Machado, J.R. Bochnia, A.S. Garcez, A. A Foggiato, Effect of photodynamic therapy potentiated by ultrasonic chamber on decontamination of acrylic and titanium surfaces, Photodiagnosis Photodyn. Ther. 27 (2019) 345–353, https://doi.org/10.1016/j.pdpdt.2019.06.011.

[26] E. Figuero, F. Graziani, I. Sanz, D. Herrera, M. Sanz, Management of peri-implant mucositis and peri-implantitis, Periodontol 2000 66 (1) (2014) 255–273, https://doi.org/10.1111/prd.12049.

[27] P. Sahrmann, F. Gilli, D.B. Wiedemeier, T. Attin, P.R. Schmidlin, L. Karygianni, The microbiome of Peri-implantitis: a systematic review and meta-analysis, Microorganisms 8 (5) (2020) 661, https://doi.org/10.3390/microorganisms8050661.

[28] C.H. Alves, K.L. Russi, N.C. Rocha, et al., Host-microbiome interactions regarding peri-implantitis and dental implant loss, J. Transl. Med. 20 (1) (2022) 425, https://doi.org/10.1186/s12967-022-03636-9.

[29] B. Zeina, J. Greenman, W.M. Purcell, B. Das, Killing of cutaneous microbial species by photodynamic therapy, Br. J. Dermatol. 144 (2) (2001) 274–278, https://doi.org/10.1046/j.1365-2133.2001.04013.x.

[30] C.A. Pereira, R.L. Romeiro, A.C. Costa, A.K. Machado, J.C. Junqueira, Jorge AO, Susceptibility of Candida albicans, Staphylococcus aureus, and Streptococcus mutans biofilms to photodynamic inactivation: an in vitro study, Lasers Med. Sci. 26 (3) (2011) 341–348, https://doi.org/10.1007/s10103-010-0852-3.

[31] M. Giannelli, G. Landini, F. Materassi, et al., Effects of photodynamic laser and violet-blue led irradiation on Staphylococcus aureus biofilm and *Escherichia coli* lipopolysaccharide attached to moderately rough titanium surface: in vitro study, Lasers Med. Sci. 32 (4) (2017) 857–864, https://doi.org/10.1007/s10103-017-2185-y.

[32] J. Marotti, P. Tortamano, S. Cai, M.S. Ribeiro, J.E. Franco, T.T. de Campos, Decontamination of dental implant surfaces by means of photodynamic therapy [published correction appears in Lasers Med Sci. 2013 May;28(3):1047], Lasers Med. Sci. 28 (1) (2013) 303–309, https://doi.org/10.1007/s10103-012-1148-6.

[33] A.A. Foggiato, D.F. Silva, R.C.F.R. Castro, Effect of photodynamic therapy on surface decontamination in clinical orthodontic instruments, Photodiagnosis Photodyn. Ther. 24 (2018) 123–128, https://doi.org/10.1016/j.pdpdt.2018.09.003.

[34] L.P. Rosa, F.C. da Silva, S.A. Nader, G.A. Meira, M.S. Viana, Antimicrobial photodynamic inactivation of Staphylococcus aureus biofilms in bone specimens using methylene blue, toluidine blue ortho and malachite green: an in vitro study, Arch. Oral. Biol. 60 (5) (2015) 675–680, https://doi.org/10.1016/j.archoralbio.2015.02.010.

[35] A. Upendran, R. Gupta, Z. Geiger, Dental infection control. StatPearls, Treasure Island (FL): StatPearls Publishing, 2023. August 8.

[36] A. Warrier, N. Mazumder, S. Prabhu, K. Satyamoorthy, T.S. Murali, Photodynamic therapy to control microbial biofilms, Photodiagnosis Photodyn. Ther. 33 (2021) 102090, https://doi.org/10.1016/j.pdpdt.2020.102090.