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Effect of photodynamic therapy potentiated by ultrasonic chamber on decontamination of acrylic and titanium surfaces

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ABSTRACT

Photodynamic Therapy (PDT) is an alternative to surface decontamination that is based on the interaction between a non-toxic photosensitizer (PS) and a light source to allow for the formation of reactive oxygen species. The objective of this study was to test a new patented device - the "Ultrasonic Photodynamic Inactivation Device" (UPID) under the patent deposit MU-BR 20.2018.00.9356-3 - for the photodynamic inactivation on contaminated acrylic plates and titanium disk. This new low cost device contains light emitting diodes (LEDs) and was built in a stainless-steel container for better light distribution. In addition, 28 waterproof red LEDs plates, with a wavelength of 660 nm were used, containing three irradiators in each plate, for which the irradiation distribution and the spectral irradiance on all 6 internal faces of this device were calculated. The effect of red LED irradiation (660 nm) methylene blue (MB) (100 μmol/L) diluted in water or 70% alcohol on three types of microorganisms: *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. In order to estimate the effects of PDI, acrylic plates and Titanium disks were contaminated by bacterial suspensions (3×10^8 CFU/mL), then treated with a solution of MB for 30 min, followed by irradiation for 30 min (0.45 J/cm^2). Microbial inhibition was evaluated by counting the number of colony forming units (CFU), compared to the control group. The results showed that the UPID promoted significant reduction ($p < 0.001$) of the microorganism when compared with the positive control. The new device promoted an effective microbial inhibition on the surfaces tested and, thus, makes possible new studies. The perspective is that this new device may be a low-cost and non-toxic alternative to the disinfection of biomedical devices, non-critical instruments and also for use in the food industry.

1. Introduction

PDT uses a photosensitizer (PS), which will be absorbed by target cells, followed by irradiation with resonant light, resulting in cell death [1–8]. Antimicrobial-PDT employs a non-toxic photosensitizer (PS), and visible light which, in the presence of oxygen, combine to produce cytotoxic oxygen species in microbes [7,9–11].

In the literature, numerous photosensitizers are used in several areas of health care [9,11–15]. However, the most effective PS in PDI belong to different groups of compounds, such as halogenated xanthenes (Bengal Rose - RB), phenothiazine (toluidine blue O - TBO and methylene blue - MB) [2].

MB is a photosensitizer belonging to the class of phenothiazine that has played an important role in microbiology, pharmacology and as a histological dye for many years [3,7,16], and its action is already well known, acting effectively on the nucleic acid [2]. In this way, this dye can be considered a good option of photosensitizer in PDT and in the inhibition and microbial control [6,8]. In addition, methylene blue shows low toxicity and no side effects [6,10], and its absorption occurs between 500 and 700 nm, with a peak at approximately 660 nm [16].

Phenothiazine derivatives such as methylene blue and toluidine blue are the most studied photosensitizers used in the treatment of and in microbial control, as in oral infections. Being low cost, the PDI procedure is easily applied to a clinical setting.

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Fig. 1. Perforated stainless steel with LED light 660 nm in ultrasonic tub - “Ultrasonic Photodynamic Inactivation Device" (UPID).

Fig. 2. Photodynamic inactivation on the surface of the plates using “UPID”.

Several clinical studies have reported the application of PDT mediated by PSs in the treatment of in the mouth and teeth surfaces [8,17,18], and decontamination [7,19–21]. Oral diseases and infections associated with mouth surfaces and implant prosthesis affects the tissues of the tooth, mucosa and periodontal tissues such as gingival, cementum, periodontal ligament and supporting bone [8,22,23]. In general, antiseptics and antibiotics often have multiple intracellular targets which may result in opportunistic infections and hypersensitivity reactions and above all can cause microbial resistance [24].

Environments and surfaces contaminated is an important factor in the transmission of infectious diseases [25]. In this way, alternative mechanisms for surface microbial control must be studied, and many are of importance in the health areas [19,26,27].

PDT is an alternative treatment for inhibition and microbial control of various surfaces, including those associated with oral health. Therefore, the aim of this study was to evaluate the antimicrobial effect of PDT using a new patented device, the “Ultrasonic Photodynamic Inactivation Device" on contaminated acrylic plates and titanium disks, and extrapolate the results to other materials and surfaces, such as surgical and aesthetic instruments, syringes, catheters, or even food packaging, laboratory and industrial equipment.

2. Methodology

2.1. Device

The "Ultrasonic Photodynamic Inactivation Device" (UPID) (MU-BR 20.2018.009356-3) was constructed using the perforated stainless steel metal basket with polypropylene lid and, for better irradiation use, the inside of the lid was covered with a thermal blanket (2 mm) of expanded polyethylene with aluminized polyester (Etaflon, São Paulo). In addition, 28 red 660 nm LED boards were used inside, containing three radiators on each 2 W plate (Rohs, China). To maintain the system, a 12 V source with 2.5 mA continuous current (Delta Electronics, China) was used. The distribution and the spectral irradiances were measured by the Luximeter (THAL-300 Instrutherm, São Paulo) the parameters were calculated in all the 6 internal faces of the device. UPID was built to be embedded in any ultrasonic bath, in this instance the model Dabi Atlante 3 L (Ribeirão Preto, São Paulo) (Fig. 1) was used.

2.2. Acrylic plate preparation

The preparation of the acrylic plates followed the modified methodology [28]. The self-curing acrylic resin (Jet, São Paulo) was separated into pots. After mixing the reagents, the blends were manipulated until they were thick and homogeneous and added to a glass mold (10 cm^2). After that, the polymer was placed in a Bubble Eliminator Pan (nº 1) (PROTÉCNI EQUIPMENT) until reaching the plastic stage, ideal for the job. The acrylic plates, which simulates the surface of dental prostheses, orthodontics appliance and similar material, was trimmed into 1 cm^2 squares and taken for the PDI tests.

2.3. Titanium disks preparation

Titanium disks nanosurface (TDNS) (SIN - National Implant System S/A) was manufactured with 6 mm diameter and 3 mm thickness. The surface of the TDNS was coated in hydroxyapatite (HA) nanocrystals with homogeneous thickness of 20 nm, according to the manufacturer. The TDNS simulate the prosthetic surface of dental implants, surgical instruments and can be used as a test body for other titanium surface instrumentals.

2.4. Photosensitizer

The Methylene Blue (MB) (Synth, São Paulo) solution was prepared at the Northern Paraná State University Biology Laboratory (UENP) Jacarezinho, Paraná, using distilled water and 70% alcohol (v/v) in 100 µmol/L [9]. The MB solution was kept in dark conditions until its use.

2.5. Proof board

This experiment used 144 acrylic plates and 144 titanium disks (TDNS) previously sterilized in autoclave (121 °C for 30 min). Randomly, 18 of each sample (acrylic and titanium) were not contaminated and served as negative control. The remainder 126 acrylic plates and 126 TDNS were divided into 3 groups (n = 42). Each group was contaminated by a type of microorganism: *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Before submitting the samples to the process of PDT, 6 samples (acrylic and titanium) of each microorganism were randomly chosen to be the positive control for each group; 6 samples (acrylic and titanium) of each microorganism were randomly chosen to be submerged in the methylene blue solution (100 µmol/L) for 20 min in 70% alcohol (v/v) and were not submitted to the PDT; 6 samples (acrylic and titanium) of each microorganism were randomly chosen to be treated in 1% peracetic acid (v/v) for 20 min for disinfection. The positive control samples were not submitted to the PDT process and the negative controls

Table 1
Results obtained for microbial growth after the PDT with MB prepared in distilled water and alcohol.

		Acrylic plates[1]		Titanium disks[2]				Acrylic plates		Titanium disks				Acrylic plates		Titanium disks	
C. albicans	Exp.	MB in water CFU	MB in alcohol 70% CFU	MB in water CFU	MB in alcohol 70% CFU	S. aureus	Exp.	MB in water CFU	MB in alcohol 70% CFU	MB in water CFU	MB in alcohol 70% CFU	E. coli	Exp.	MB in water CFU	MB in alcohol 70% CFU	MB in water CFU	MB in alcohol 70% CFU
	1	41	0	0	0		1	0	0	0	0		1	0	0	0	0
	2	71	0	0	0		2	2	2	0	0		2	0	0	0	0
	3	90	3	0	0		3	27	0	0	0		3	0	0	0	0
	4	158	0	0	0		4	1	1	0	0		4	0	0	0	0
	5	225	0	0	0		5	0	2	0	0		5	0	0	0	0
	6	55	0	0	0		6	3	0	0	0		6	0	0	0	0
	7	150	0	0	0		7	0	0	0	0		7	0	0	0	0
	8	454	1	0	0		8	0	1	0	0		8	0	0	0	0
	9	519	2	0	0		9	1	0	0	0		9	0	0	0	0
	10	157	0	0	0		10	2	0	0	0		10	0	0	0	0
	11	91	0	0	0		11	0	0	0	0		11	0	0	0	0
	12	187	0	0	0		12	1	1	0	0		12	0	0	0	0
	C+	25312		23319			C+	62304		41304			C+	56017		59211	
	C+	24299		22299			C+	61299		39299			C+	76335		63015	
	C+	25301		19351			C+	55311		41311			C+	66128		60292	
	C+	25297		30897			C+	59310		38310			C+	56587		57014	
	C+	21315		29031			C+	68351		35351			C+	69139		66203	
	C+	19325		33032			C+	59378		42378			C+	70279		60753	
	C-	0		0			C-	0		0			C-	0		0	
	C-	0		0			C-	0		0			C-	0		0	
	C-	0		0			C-	0		0			C-	0		0	
	C-	0		0			C-	0		0			C-	0		0	
	C-	0		0			C-	0		0			C-	0		0	
	C-	0		0			C-	0		0			C-	0		0	
	Alcohol[3]	1022		912			Alcohol[3]	822		212			Alcohol[3]	372		192	
	Alcohol	998		1071			Alcohol	698		171			Alcohol	318		378	
	Alcohol	1051		1101			Alcohol	851		320			Alcohol	411		120	
	Alcohol	801		992			Alcohol	501		492			Alcohol	519		292	
	Alcohol	1098		1015			Alcohol	708		415			Alcohol	324		216	
	Alcohol	951		790			Alcohol	459		390			Alcohol	295		199	
	Per. acid	0		0			Per. acid	0		0			Per. acid	0		0	
	Per. acid[4]	0		0			Per. acid[4]	0		0			Per. acid	0		0	
	Per. acid	0		0			Per. acid	0		0			Per. acid	0		0	
	Per. acid	0		0			Per. acid	0		0			Per. acid	0		0	
	Per. acid	0		0			Per. acid	0		0			Per. acid	0		0	
	Per. acid	0		0			Per. acid	0		0			Per. acid	0		0	

[1] The acrylic plates were prepared according to item 2.2.
[2] The titanium plates were prepared according to item 2.3.
[3] Samples submerged for 20 min in methylene blue solution (100 μmol/L) diluted in 70% alcohol (v/v) without passing the PDT procedure.
[4] Samples disinfected with 1% Peracetic Acid (v/v) for 20 min.

Table 2
Statistical analysis of CFU values in culture using acrylic plates.

Group	Control -	Control +	After photodynamic inactivation (MB in water)	Mann-Whitney Test[3]	After photodynamic inactivation (MB in alcohol 70%)	Mann-Whitney Test[4]
C. albicans	All values equal to zero (n = 6)	Mean = 23474,83 SD = 2553.25 (n = 6)	Mean = 183.17 SD = 152.76 (n = 12)	$p < 0.001$	Mean = 0.50 SD = 1.0 (n = 12)	$p < 0.001$
S. aureus	All values equal to zero (n = 6)	Mean = 60992.16 SD = 4328.47 (n = 6)	Mean = 1.00 SD = 1.04 (n = 12)	$p < 0.001$	Mean = 0.58 SD = 0.79 (n = 12)	$p > 0.05$
E. coli	All values equal to zero (n = 6)	Mean = 65747.50 SD = 8035.84 (n = 6)	Mean = 0.00 SD = 0.00 (n = 12)	$p < 0.001$	Mean = 0.00 SD = 0.00 (n = 12)	$p > 0.05$
		Kruskal-Wallis test[1]	$p < 0.05$	*Kruskal-Wallis test*[2]	$p > 0.05$	

[1] Significance level of the Kruskal-Wallis test for the comparison CFU after PDI between the solutions of methylene blue in water.
[2] Significance level of the Kruskal-Wallis test for the comparison CFU after PDI between the solutions of methylene blue in alcohol 70%.
[3] Significance level of the Mann-Whitney Test for comparison with the control +.
[4] Significance level of the Mann-Whitney Test for comparison with the solutions of Methylene Blue (water and alcohol 70%).

were autoclaved. The other 24 samples in each group were submitted to PDT and, at the end of the laboratorial processes, results were assessed with a sterile swab strewn in a petri dish for Colony-Forming Unit (CFU).

2.6. Microorganisms

The microorganisms used were *C. albicans* ATCC 10231, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. They were individually added into 50 microliters of sterile BHI broth and growth was done in microaerobic stove (Tecnal TE-399) at 36 °C for 24 h, until stationary stage. Afterwards, the bacterial solutions were diluted using a McFarland scale in equivalent to 3×10^8 CFU/mL.

The samples in each group (acrylic or titanium) were placed in a glass container (10 x 20 cm) containing 300 mL for each microbial strain respectively diluted in stationary stage diluted in phosphate buffered saline (PBS) (McFarland scale-3×10^8 CFU/mL) and left for 15 min in room temperature.

2.7. Photodynamic inactivation for PDT

After the sample contamination process, PDT was applied for microbial inhibition. The plates were divided into two groups (24 plates for each) placed in polypropylene bags containing 200 mL of methylene blue (100 μmol/L), diluted in distilled water and 70% alcohol (v/v),for 20 min better PS absorption. Afterwards, UPID (Fig. 2) was used for PDT treatment for 30 min (energy density of 0.45 J/cm^2), according to Garcez [9]. The samples were removed, after left in sterile chapel for 10 min and swabbed in sterile PBS. Afterwards, the whole surface of a 50 mm diameter petri dish containing BHI was scored. The procedure aimed at CFU, according to the method proposed by Jett [29]. The dishes were placed in a microaerophilic incubator with 5% oxygen, 10% carbon dioxide and 80% nitrogen incubated for 24 h at 37 °C. The CFU counting was done with a Phoenix CP608 magnifying glass (Phoenix Industry and Commerce of Scientific Equipment Ltd.). All the samples were handled in a contamination free area (laminar flow and Bunsen burner) during the preparation of culture, irradiation and measuring of the inhibition area and all material involved were previously autoclaved.

2.8. Statistical analysis

The statistical analysis was accomplished with the *Statistical Package for the Social Sciences* (SPSS) – Version 25. The data normality was tested with the Shapiro-Wilk Test. Once the CFU variable had non-normal distribution in groups of microorganisms, (Shapiro-Wilk Test: $p < 0.001$), Mann-Whitney Te*st* was used for comparing the CFU average of this group with the positive control and different types of methylene blue solution. For the result analysis of the differences amongst the three groups, after PDT, the Kruskal-Wallis nonparametric test was used. In the statistical test a level of 5% meaningfulness was considered. Thus, the differences were considered statistically meaningful if the meaningfulness was less than 0.05 ($p < 0.05$). The graph was made in the Origin Pro-Version 9.1.

3. Results

Table 1 shows the results obtained in the CFU counting of the microbial cultures of *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The statistical analyses are in Table 2. In the positive control group, initial count of *C. albicans* was 23474.83 ± 2553.25 CFU/mL (Fig. 3a), *S. aureus* was 60992.16 ± 4328.47 CFU/mL (Fig. 3d) and *E. coli* was 65747.50 ± 8035.84 CFU/mL (Fig. 3g). After PDT, the CFU/mL average value was significantly lower ($p < 0.001$). The Table 3 shows the statistical analysis for TDNS, in addition the CFU values of “negative

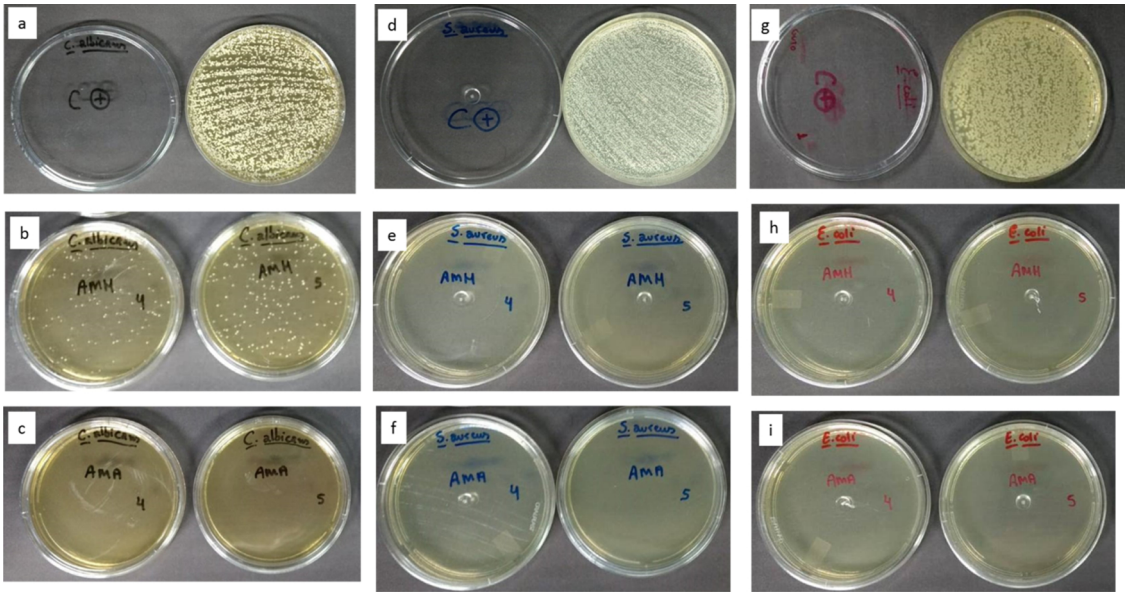


Fig. 3. Culture plates with different numbers of CFU presented in acrylic plates: **a)** *Candida Albicans* – Control +; **b)** *Candida Albicans* – PDI with MB in water; **c)** *Candida Albicans* – PDI with MB in alcohol 70%; **d)** *Staphylococcus aureus* – Control +; **e)** *Staphylococcus aureus* – PDI with MB in water; **f)** *Staphylococcus aureus* – PDI with MB in alcohol 70%; **g)** *Escherichia coli* – Control +; **h)** *Escherichia coli* – PDI with MB in water; **i)** *Escherichia coli* – PDI with MB in alcohol 70%.

control" equaled to zero in the three groups. In the group *C. albicans* the positive control was 26321.50 ± 2553.25 CFU/mL (Fig. 4a), the *S. aureus* was 39658.86 ± 2585.10 CFU/mL (Fig. 4d) and *E. coli* was 61081.33 ± 3185.67 CFU/mL (Fig. 4g). After PDT, the CFU average value was significantly inferior ($p < 0.001$), the results being statistically identical ($p > 0.05$) (Graphic 1 and 2). After PDT the CFU values were virtually zero, these results are equal to the negative control (autoclaved samples) and the samples treated with 1% peracetic acid (v/v). Samples treated only with the methylene blue solution in 70% alcohol (v/v) showed microbial inhibition (Table 1). However, these are statistically different from the results of PDT ($p < 0.05$).

The results demonstrate that both solutions of methylene blue, water and alcohol, showed an effective antimicrobial effect when compared to the positive controls ($p < 0.001$) and with 1% peracetic acid (v/v). However, for *C. albicans,* when grown on acrylic plates, methylene blue in alcohol solution was significantly more effective ($p < 0.001$). The other results obtained were significantly similar ($p > 0.05$).

4. Discussion

The results obtained in this study demonstrated that the patent MU-BR 20.2018.009356-3 "Ultrasonic Photodynamic Inactivation Device" (UPID) and the concentration of MB used (100 µmol/L), both in water and in ethanol (70%), proved to be effective for the decontamination of acrylic plates and titanium disks surfaces under the conditions tested. Microbial decontamination was effective against yeast, gram-positive and gram-negative cultures. All microorganisms studied are found in the human microbiota, including the buccal microbiota [22,23,30,31] and are often associated with different diseases [32–36].

Several applications are known for acrylic oral health [37–39] and titanium implants, titanium prosthesis and titanium instruments [40–42], for example a clinical report of implant-supported fixed complete dental prosthesis with titanium milled molars [43]. However, many diseases may be associated [44–47]. *Candida albicans* and *Staphylococcus aureus* are often co-isolated in cases of biofilm associated infections [32]. According to the same authors [32], this yeast cause systemic disease through morphological switch from the rounded yeast to the invasive hyphal form, and *S. aureus is* able to invade host tissue and disseminate via adherence to the invasive hyphal elements of *Candida albicans*. The authors [34] affirm that these microorganisms, when acting in synergic form, increase significantly biofilm maturation. This joint action and the other microorganisms of the oral mucosa can cause numerous pathologies with systemic results. In another situation, contamination of dental implants constituted by titanium could result in increased frequency of peri-implantitis and subsequent implant loss. Cross-sectional studies on implant-treated patients showed that peri-implant mucositis occurred in 80% of patients and in 50% of implant sites. Peri-implantitis was identified in 28–56% of patients and in 12–43% of implant sites [48,49]. Thus, proper decontamination prior to the implantation procedure is important and necessary.

Microbial surface control that could evolve into an infectious disease usually consists of the use of products that over time can cause microbial resistance, such as chemical and physical agents, antiseptics or even antibiotics. In this way, an alternative in microbial surface control demonstrates extreme importance, which can reduce cross-infections and microbial diseases in general. Recent studies [19] demonstrated the effect of photodynamic therapy on surface decontamination in clinical orthodontic instruments and showed that Photodynamic Inactivation Device (PID), a new patented device - Patent Deposit MU-BR20.2017.002297-3, caused a significant reduction ($p < 0.05$) of the microbial charge (gram-positive and gram-negative bacteria) in the orthodontic instruments.

The great advantage of PDT is that it has an action against all types of microorganisms, including eukaryotic cells, such as yeast. The results presented in this work were extremely satisfactory, as they were able to drastically reduce the microbial load of the tested surfaces of both acrylic plates and titanium disks. The results can be extrapolated to other instrumentation surfaces and health appliances such as catheters, syringes and gloves or even manicure instruments and laboratory supplies. The data obtained were significant because *C. albicans* and other yeasts have shown a greater resistance to antimicrobial actions, when treated with PDT, compared to gram-positive bacteria. This factor might happen due to the presence of a nuclear membrane in the structure of yeasts, and also the greater cell size and reduced number of target of singlet oxygen [50]. Another interesting point related to this yeast is indiscriminate use of antifungals that has led to resistance of *Candida* sp. This fact requires new treatment alternatives for fungal contamination in removable prostheses, which is related to fungal infections such as Candidosis or prosthetic stomatitis.

Table 3
Statistical analysis of CFU values in culture using TDNS.

Group	Control -	Control +	After photodynamic inactivation (MB in water)	*Mann-Whitney Test*[3]	After photodynamic inactivation (MB in alcohol 70%)	*Mann-Whitney Test*[4]
C. albicans	All values equal to zero (n = 6)	Mean = 26321.50 SD = 2553,25 (n = 6)	Mean = 0.00 SD = 0.00 (n = 12)	$p < 0.001$	Mean = 0.00 SD = 0.00 (n = 12)	$p > 0.05$
S. aureus	All values equal to zero (n = 6)	Mean = 39658.86 SD = 2585.10 (n = 6)	Mean = 0.00 SD = 0.00 (n = 12)	$p < 0.001$	Mean = 0.00 SD = 0.00 (n = 12)	$p > 0.05$
E. coli	All values equal to zero (n = 6)	Mean = 61081.33 SD = 3185.67 (n = 6)	Mean = 0.00 SD = 0.00 (n = 12)	$p < 0.001$	Mean = 0.00 SD = 0.00 (n = 12)	$p > 0.05$
		Kruskal-Wallis test[1]	–	*Kruskal-Wallis test*[2]	–	

[1] Significance level of the Kruskal-Wallis test for the comparison CFU after PDI between the solutions of methylene blue in water.
[2] Significance level of the Kruskal-Wallis test for the comparison CFU after PDI between the solutions of methylene blue in alcohol 70%.
[3] Significance level of the Mann-Whitney Test for comparison with the control +.
[4] Significance level of the Mann-Whitney Test for comparison with the solutions of Methylene Blue (water and alcohol 70%).

The application of photodynamic therapy (PDT) has been investigated in the inactivation of microorganisms pathogenic to the human host [17,50,51]. The gram-negative microorganisms also demonstrate resistance to PDT due to low penetration of MB, as these bacteria have an outer membrane [52]. The results obtained corroborate with the literature [51] that evaluate specific effects of PDT using methylene blue as photosensitizer and low-power laser irradiation on the viability of single, dual and three-species biofilms formed by *C. albicans*, *S. aureus* and *Streptococcus mutans*. The results above showed significant decreases in the viability of all microorganisms were observed for biofilms exposed to PDT mediated by MB dye. In another study [55] the effectiveness of microbial surface decontamination in dental implant by PDT was proved and the authors affirmed that photodynamic therapy can be considered an efficient method for reducing bacteria on implant surfaces, when compared with chlorhexidine. The authors [21] studied antimicrobial effect of photodynamic therapy using methylene blue and diode laser on biofilm attached to sandblasted and acid-etched surface of titanium, the results showing that PDT demonstrated significant differences of untreated groups ($p < 0.05$). The results were similar to those found in this study, in which the surface of titanium was decontaminated by the action of PDT.

Cardote et al [53] working with cationic corrole derivatives exhibited demonstrated antimicrobial action at micromolar concentration against Gram-negative bacterium strain. In another study [54] was demonstrated the antimicrobial action of the compound chlorophyll based photosensitizer (PS) Zn(II)chlorin e_6 methyl ester (Zn(II)e_6Me) against *Enterococcus faecalis* and *Candida albicans* in biofilm by action photodynamic therapy (red light - 627 nm, 75 mW, 3150 J/cm^2). These new compounds could be applied effectively and increase the antimicrobial action in the patent (MU-BR 20.2018.009356-3 “Ultrasonic Photodynamic Inactivation Device" - UPID) presented in this work.

The positive results of UPID in this study also corroborate the antibacterial effect reported in previous studies with *S. aureus, S. mutans* (gram-positive) and *E. coli* (gram-negative) [19,51,52,56,57]. Microorganisms can affect human health in a number of ways, because human tissues and mucous membranes are inhabited by more than 10^4 microorganisms. Of these microorganisms the *C. albicans, S. aureus* and *E. coli* could be the etiological agents of various pathologies, including oral infections [22,34,58,59]. The presence of pathogenic microorganisms on surfaces of prostheses, instruments, implants and other places can cause various health problems, for example Prosthetic Stomatitis [60–63].

This study evaluated the antimicrobial action against yeast, gram-positive and gram-negative bacteria in surfaces of acrylic plates and titanium disks using the new patented device (UPID), by photodynamic therapy. Effective microbial control was reported by the reduction of the microbial load on the surfaces both of acrylic plates and of titanium disks.

The main problem in health actions is to avoid cross-contamination, which often occurs due to instruments or tools used by the professional [22,23,31]. In this way, the clinical environment is a suitable means of exposing professionals and their patients to biological risks [36,64,65].

Based on current results, it could be concluded that UPID was effective for PDT against *C. albicans, S. aureus* and *E. coli*, all microorganisms associated with diseases, general infections and oral infections, for example, microorganisms directly related to prosthetic stomatitis and peri-implantitis infections. The results obtained demonstrated equivalence to the autoclaving procedure or the disinfection with 1% peracetic acid (v/v), for the samples tested. Therefore, caution is required when extrapolating the present findings to clinical practice. These require further studies, especially when dealing with yeast and gram-negative bacteria, considering that these microorganisms are more resistant to MB penetration. This is the advantage of using alcohol (70%), since it has the ability to dilute the plasma membrane.

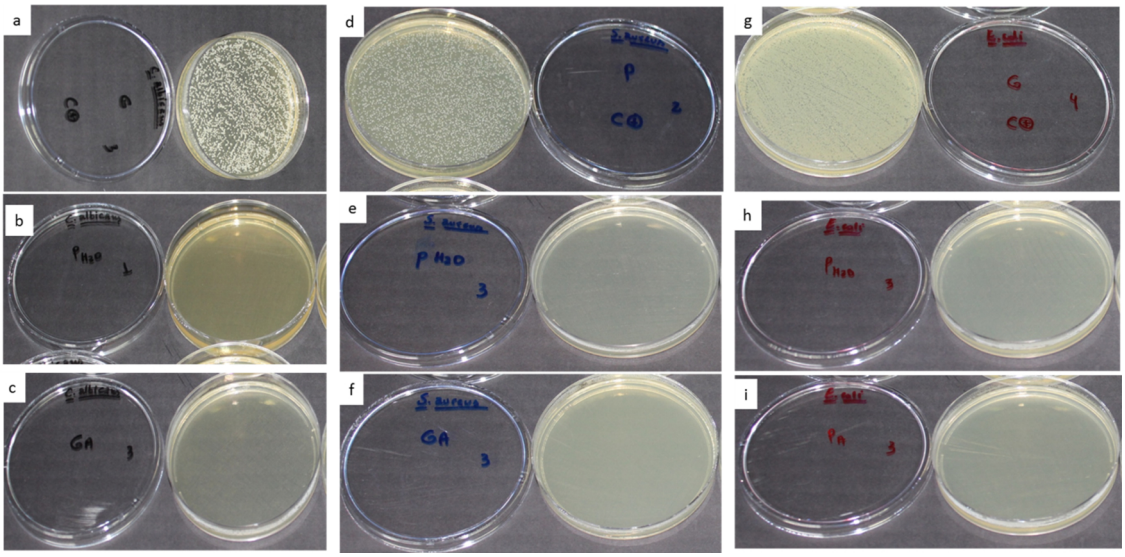
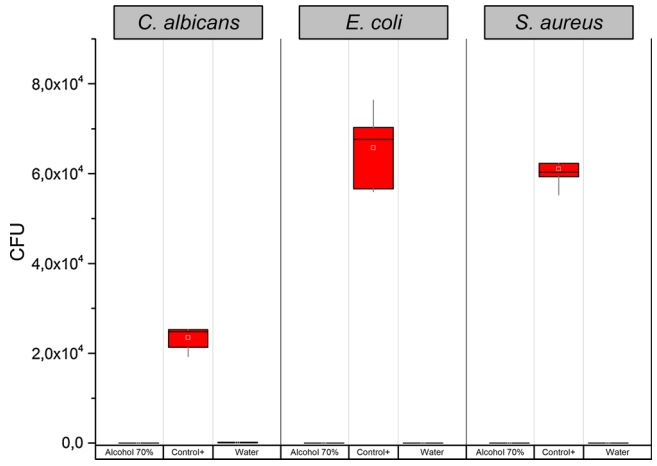
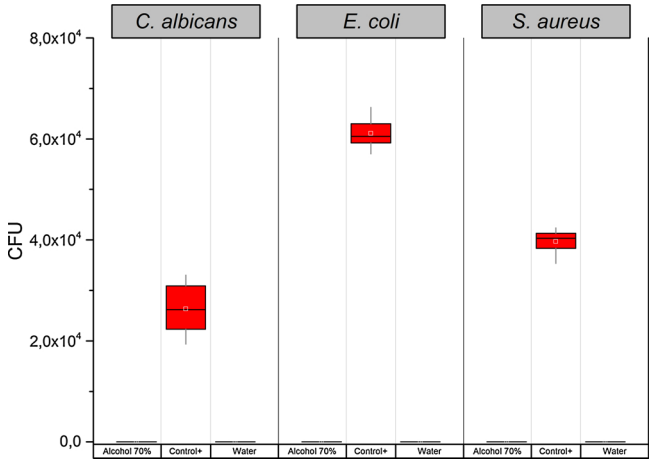


Fig. 4. Culture plates with different numbers of CFU presented in TDNS: **a)** *Candida Albicans* – Control +; **b)** *Candida Albicans* – PDI with MB in water; **c)** *Candida Albicans* – PDI with MB in alcohol 70%; **d)** *Staphylococcus aureus* – Control +; **e)** *Staphylococcus aureus* – PDI with MB in water; **f)** *Staphylococcus aureus* – PDI with MB in alcohol 70%; **g)** *Escherichia coli* – Control +; **h)** *Escherichia coli* – PDI with MB in water; **i)** *Escherichia coli* – PDI with MB in alcohol 70%.



Graphic 1. Boxplot with values of Colony Formation Unit (CFU) for microorganisms evaluated after photodynamic inactivation with MB solution prepared using distilled water or 70% alcohol (30 min - 0.45 J/cm^2) on acrylic plates.



Graphic 2. Boxplot with values of Colony Formation Unit (CFU) for microorganisms evaluated after photodynamic inactivation with MB solution prepared using distilled water or 70% alcohol (30 min - 0.45 J/cm^2) on titanium disks (TDNS).

5. Conclusion

The results presented in this study demonstrate that the "Ultrasonic Photodynamic Inactivation Device" (UPID) referring to the patent "MU-BR 20.2018.009356-3" were effective for microbial control on acrylic and titanium surfaces against yeast, gram-positive and gram-negative bacteria. These results, along with several studies in the literature, suggest good perspectives for the formulation of adequate clinical protocols for microbial control and, thus, propose an atoxic and low cost alternative to disinfection of biomedical tools as noncritical instruments and eventually be used in laboratories, aesthetic, dental, medical clinics as well as for food and general industry.

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