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Efficacy of phthalocyanine dye in antimicrobial debridement by photodynamic therapy as an adjunct to mechanical management of peri-implantitis: an *in vitro* study

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Abstract

Objective: The aim of the present *in vitro* study was to evaluate the efficacy of phthalocyanine dye in photodynamic therapy (PDT) as an adjunct to mechanical debridement in the management of peri-implantitis.

Methods: *S. aureus, F. nucleatum* and *P. gingivalis* were coated on titanium (Ti) discs and were divided in control and test groups. In control group, mechanical debridement + laser was performed; and in test group, PDT with hydroxyaluminium phthalocyanine (AlPcOH) dye as photosensitizer was used. Bacterial colony counts and surface hardness were assessed post-operatively.

Results: There was a significant difference in the bacterial count reduction at different time intervals in the test group (p=0.01), when compared to the control group; no significant difference was observed in surfaces hardness (*p=0.7*).

Conclusion: This present *in vitro* study concluded that PDT with AlPcOH dye was effective in eradicating *S. aureus, F. nucleatum* and *P. gingivalis* on Ti discs. This study also concluded that the PDT with photosensitizer can be used as an adjunct in management of peri-implantitis.

Keywords: Phthalocyanine dye. Photodynamic therapy. Peri-implantitis. Bacterial count. In vitro study.

INTRODUCTION

Peri-implantitis is defined as a plaque-associated pathologic condition occurring in tissue around dental implants, characterized by inflammation in the peri-implant mucosa and subsequent progressive loss of supporting bone (Berglundh *et al*., 2018). Microbial colonization and biofilm formation on implant surfaces play a pivotal part in the development and progression of peri-implantitis (Subramani *et al*., 2009). Being a poly-microbial anaerobic infection, peri-implantitis has been found to harbor a spectrum of periodontopathic bacteria (Charalampakis *et al*., 2012).

During biofilm formation, *S. aureus* acts as an early colonizer, creating a favorable environment for the adhesion and colonization of late colonizers (Persson *et al*., 2014). Therefore, it is essential to effectively eradicate *S. aureus* biofilm in peri-implantitis. *F. nucleatum* also plays a role in biofilm formation, host infection and host response in peri-implantitis, by stimulating the expression of IL-1β, causing the typical clinical symptoms of peri-implant diseases (Virto *et al*., 2022). *P. gingivalis* has been confirmed as a critical pathogen in peri-implantitis, represented by inflammation of peri-implant soft and hard tissues. This may result in colonization of bacteria on implant surfaces (Mahato *et al*., 2016; Ata-Ali *et al*., 2011) resulting in considerably high prevalence of peri-implantitis (20% to 56%) (Mombelli *et al*., 2012).

In vitro studies have demonstrated that *S. aureus* has a strong affinity to titanium surfaces. Thus, *S. aureus* infection may be of importance in the development of peri-implantitis induced by bacterial infection. According to the results of Salvi *et al*. (2008), which showed high positive (80%) and negative (90%) predictive values, *P. gingivalis* is also associated with peri-implantitis and periodontitis, respectively, and is known to evade the host response and promote tissue destruction.

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Various protocols have been proposed to treat peri-implantitis. These protocols comprise of non-surgical and surgical therapy. Lasers can be used in decontamination of implant surfaces. The most frequently used include diode, erbium lasers and CO_2, due to their hemostatic properties, selective calculus ablation, and bactericidal effects. An alternative approach to dental implant decontamination is the association of the conventional treatment with photodynamic therapy (PDT) (Hofauer *et al*., 2019).

There is a growing interest in the development of other forms of treatment such as antimicrobial photodynamic therapy (aPDT) and use of lasers in decontamination of implant surface (Alvarenga *et al*., 2019). The antimicrobial effect of PDT is based on particular interaction of a photosensitizer or specific dye that attaches to the surface of microorganisms and the radiation emitted by a laser source.

Phthalocyanines are photosensitizers derived from synthetic porphyrins of high quantum yield ($\Phi\Delta > 0.4$) and absorption in red electromagnetic spectrum (600–900 nm), which corresponds to the therapeutic range of PDT. As hydrophobic photoactive drugs, phthalocyanines interact easily with cell membranes, increasing its efficiency to generate singlet oxygen with high binding affinity to microbes, which are known to show promising antimicrobial photodynamic therapeutic properties (Melo *et al*., 2018). Aluminum phthalocyanines have been extensively employed in antimicrobial PDT as effective photosensitizers, as they are least cytotoxic due to its lipophilic nature (Reis *et al*., 2014). Its efficacy has been demonstrated both *in vitro* and *in vivo* studies, particularly on *S. aureus* in suspensions and biofilms (Carvalho *et al*., 2011).

Aluminum phthalocyanines (AlPc) that have been developed have shown good inactivation of various microbial pathogens. The amphiphilic character of photosensitizer is an important property in photodynamic inactivation of microbes. This property may be enhanced by cationic charges, the number of these cationic charges, distribution of cationic charge, and the substituted group at lipophilicity of photosensitizer molecule. Photosensitizers with one or more cationic groups have shown to be effective in photoinactivation of both gram-positive and gram-negative bacteria (Nyamu *et al*., 2018).

Various studies have showed the anti-microbial effects of AlPc as photosensitizers in periodontitis, but there is limited data regarding its efficiency in peri-implantitis. So, this study was designed to observe *in vitro* the effect of PDT using AlPc dye as a photosensitizer on biofilms of *S. aureus*, *P. gingivalis*, and *F. nucleatum* coated on titanium discs.

MATERIALS AND METHODS

Trial design

The study was designed as an *in vitro* study to evaluate bacterial count and surface hardness of titanium discs after applying a novel photosensitizer: hydroxyaluminum phthalocyanine (AlPcOH)+LASER+SRP.

Sample size calculation

A sample size of 10 per group was calculated (n=20) for an effect size of 1.1, with a probability of error of 0.05 at a statistical power of 0.80.

Preparation of hydroxyaluminum phthalocyanine gel solution

AlPc was dissolved in 100mL of distilled water to prepare an initial 65.5 mg/mL AlPc stock solution. The AlPc stock solution was further diluted in balanced saline solution (OmniSol®) to achieve the final AlPcOH dye concentration of 3.75%, which was used in the trial (Fig. 1).

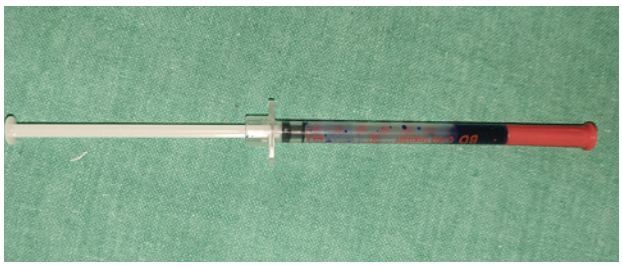

Figure 1. Hydroxy aluminum phthalocyanine (AlPcOH) dye.

Bacterial culture and biofilm formation

S. aureus, *P. gingivalis* and *F. nucleatum* strains were grown overnight on agar medium and then inoculated to obtain bacterial suspension. Thereafter, suspension was diluted with phosphate buffered saline (PBS) mixed by repeated vortexing and then adjusted to 1×10^8 CFU/mL using UV spectrophotometer. Fresh lyophilized *P. gingivalis* and *F. nucleatum* were used and rehydrated in brain heart infusion (BHI) broth and peptone yeast glucose (PYG) medium (American type culture collection Manassas, USA, ATCC33277, ATCC25586), respectively, and incubated in an anaerobic jar at <1% O_2 and 9-13% CO_2 at 37°C. All the strains were sub-cultured twice before exposure to light. The bacterial concentration after 24hrs incubation was standardized by dilution with sterile broth to OD 650nm = 0.45, equivalent to $\cong 5\times10^6$ colony forming units (CFU). The incubation time of *S. aureus, P. gingivalis* and *F. nucleatum* biofilms was set and then the titanium discs were incorporated with these three microorganisms. Then the bacterial count was evaluated at different time intervals, as follows: 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 240hrs, 264hrs, 288hrs, 336hrs, 380hrs.

Ti specimen preparation

Grade IV polished Ti discs (n=20) were used in this study. Each disc had a diameter of 10.0mm and thickness of 1.0 mm. Ti discs were sequentially sonicated with acetone, absolute ethanol and deionized water for 15 min, and were rinsed with distilled water and autoclaved for 15 min at 121°C (Fig. 2).

Treatment protocol

After contamination with *S. aureus*, *F. nucleatum* and *P. gingivalis*, Ti discs were rinsed with phosphate buffered saline (PBS) three times, to remove loose bacteria around Ti discs, before decontamination procedure. Ti discs (20 polished) were randomly divided into two decontamination groups. Ti discs receiving mechanical debridement served as a control group, and discs receiving the photosensitizer acted as test group specimens. In the test group, Ti discs were treated with a combination of AlPcOH+LASER+Scaling and Root Planning (SRP), while control group specimens received treatment with LASER+SRP. AlPcOH dye was applied with a blunt needle starting from the center to periphery, and left in contact for 30 minutes. Then the area of the titanium surface was irrigated with saline solution and irradiated with diode laser at wavelength 800nm with an output power of 100mW for 10 seconds.

Viability assessment

The antibacterial activity of the specimens was assessed against *S. aureus*, *P. gingivalis* and *F. nucleatum*. The biofilm growth in 12-well microtiter plates was estimated by using the crystal violet assay, a dye specific to biofilm biomass, in each plate, which contained one Ti disc coated by a different type of culture media. After treatment, disc was transferred into a test tube containing 1 mL PBS and vortexed for 1 min, in order to detach residual biofilm from Ti discs. The samples were serially diluted in PBS solution and plated by a spiral plater. *S. aureus*, *F. nucleatum* and *P. gingivalis* biofilms were plated on LB agar plate and incubated anaerobically at 37°C for 36 h. Finally, the number of colonies from the proper range was calculated for analysis of disinfection (Fig. 3).

Figure 2. A) Titanium discs. B) Discs with test material. C) Discs with laser treatment.

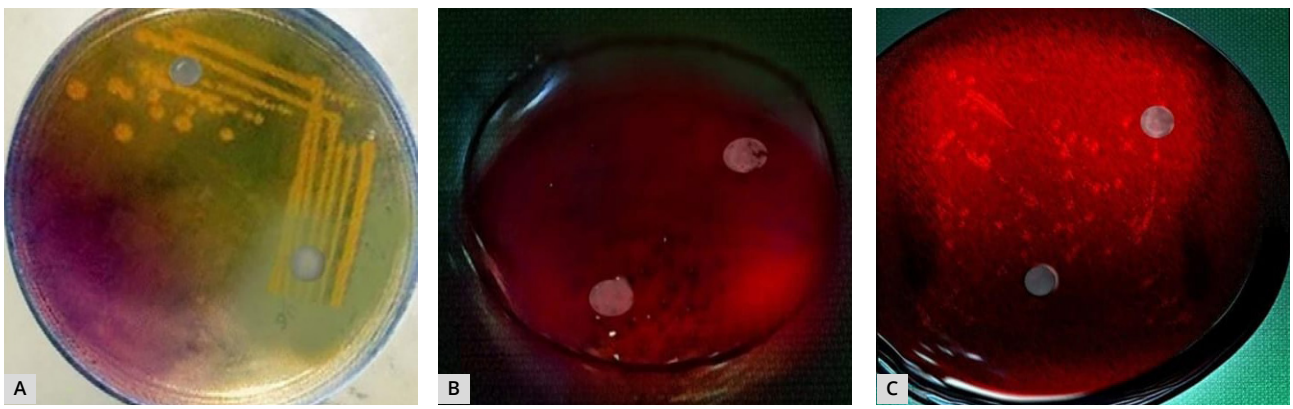

Figure 3. A) Disc coated with S. aureus. B) Disc coated with P. gingivalis. C) Disc coated with F. nucleatum.

Assessment of implant surface hardness

The surface hardness was determined by using Vickers hardness test device, measured by using a 500-g load and a 15-s loading time (Micromet 2100©, Buehler, India).

STATISTICAL ANALYSIS

All the variables in the study were subjected to statistical analysis, obtained using the average and standard deviation of the colonies count (in CFU/ml). Calculation of percentage (descriptive statistics) was made using the Friedman test, Mann-Whitney U test, Wilcoxon signed rank test, using SPSS software 20 package. The hypothesis verification of equal variances (p<0.001) was considered as statistically significant. Vickers hardness means (VHN) and standard deviations were analyzed statistically by Kruskal-Wallis non-parametric test, at 5% significance level.

Observations and results

This *in vitro* study was carried out to determine the efficacy of hydroxyaluminium phthalocyanine (AlPcOH) dye on different microorganisms coated on Ti surfaces. The bacterial colony count and surface hardness were evaluated before and after application of AlPcOH dye as photosensitizer.

Intragroup comparison

S. aureus growth of test &control groups at various time intervals

The mean values of bacterial colony count in test group were 406834.7, 1618412.9, 1741388.76, 2199773.4, 2181511.43, 2561102.5, 2774643.78, 3705239.9, 4163413.7, 4505351.24; and in control group were 300315.8, 921181.4, 1826737.38, 2171695.4, 2486776.4, 2912439.27, 4512341.6, 4722922.12, 4818033.6 (Table 1) at various time intervals. It was observed that the p-value was highly significant (p <0.001).

P. gingivalis growth of test & control groups at various time intervals

The mean values of bacterial colony count in test group were 31799.00, 44765.69, 149211.10, 136587.49, 441619.99, 624237.57, 896603.2, 125719.9, 5626497.39, 7870870.78; and in control group were 31799.00, 44765.69, 149211.10, 136587.49, 441619.99, 624237.57, 896603.2, 125719.9, 5626497.39, 7870870.78 (Table 2) at various time intervals. It was observed that the p-value was highly statistically significant (p<0.001).

F. nucleatum growth of test & control groups at various time intervals

The mean values of bacterial colony count in test group were 18483.42, 65603.6, 75435.6, 306146.7, 512754.2, 858376.8, 1972109.9, 2484429.0, 3563766.6, 4801315.0; and in control group were 11679.8, 31781.6, 67536.2, 413815.1, 1640872.0, 3935379.8, 4628975.3, 5549451.2, 3951686.8, 5755508.8 (Table 3) at various time intervals. It was observed that the p-value was highly statistically significant (p<0.001).

Intergroups comparison

S. aureus growth at various time intervals in test and control groups

For the mean bacterial count in *S. aureus* group comparison, no significant difference was observed at time intervals between 48hrs (p = 0.940) and 72hrs (p = 0.199), and the remaining time intervals showed highly statistically significant p-value (p=0.001) (Table 1).

P. gingivalis growth at various time intervals in test and control groups

For the mean bacterial count of intergroups comparing of *P. gingivalis* of both test and control groups at various time intervals, the p-value was highly statistically significant (p=0.001) (Table 2).

Table 1. Intergroup comparison of *S. aureus* between test and control groups.

Intervals	TEST GROUP		CONTROL GROUP		U VALUE	P-VALUE
	MEAN	SD	MEAN	SD	**0.000**	< 0.001*
24 hrs	406834.7	8401.3	300315.8	1159.2	0.000	< 0.001*
48 hrs	1618412.9	49921.85	921181.4	11096.2	49.0	0.940
72 hrs	1741388.6	355729.5	1826737.8	59891.4	33.00	0.199
96 hrs	2199773.4	46166.15	2171695.4	58562.6	0.000	< 0.001*
120 hrs	2181511.3	110836.6	2486776.4	2999.1	0.000	< 0.001*
240 hrs	2561102.5	82475.42	2912439.7	1171.7	0.000	< 0.001*
264 hrs	2774643.8	99006.43	4512341.6	3049.7	0.000	< 0.001*
288 hrs	3705239.9	104583.8	4549888.2	937.9	0.000	< 0.001*
336 hrs	4163413.7	52046.78	4722922.2	4867.6	0.000	< 0.001*
380 hrs	4505351.4	66264.8	4818033.6	1390.1	0.000	< 0.001*

Mann-Whitney U test (significant for p<0.05*).

Table 2. Intergroup comparison of *P. gingivalis* between test and control groups.

Intervals	TEST GROUP		CONTROL GROUP		U VALUE	P-VALUE
	MEAN	SD	MEAN	SD		
24 hrs	24278.30	453.09	31799.00	907.06	0.000	<0.001*
48 hrs	32808.46	529.01	44765.69	934.44	0.000	<0.001*
72 hrs	56433.04	590.46	149211.10	223936.6	0.000	<0.001*
96 hrs	98491.84	2291.85	136587.49	5027.30	0.000	<0.001*
120 hrs	150723.78	5790.21	441619.99	4124.3	0.000	<0.001*
240 hrs	371133.49	7312.61	624237.57	6286.4	0.000	<0.001*
264 hrs	1035030.54	1546957.87	896603.2	5241.2	10.00	0.002*
288 hrs	893223.60	2551.03	125719.9	3243.09	0.000	<0.001*
336 hrs	3281788.52	4880013.20	5626497.39	62790.28	10.000	0.002*
380 hrs	4869164.7	7754.7	7870870.78	63843.35	0.000	<0.001*

Mann-Whitney U test (significant for p<0.05*).

F. nucleatum growth at various time intervals in test and control groups

For the mean bacterial count of intergroups comparison of *F. nucleatum* of both test and control groups at various time intervals, the *p*-value was highly statistically significant ($p = 0.001$) (Table 3).

Intergroup comparison of surface hardness

For the mean values of surface hardness in test group (362.78), the *p*-value showed no significant difference ($p=0.07$) (Table 4).

Table 3. Intergroup comparison of *F. nucleatum* among test and control groups.

Intervals	TEST GROUP		CONTROL GROUP		U VALUE	P-VALUE
	MEAN	SD	MEAN	SD		
24 hrs	18483.42	627.2	11679.8	5473.8	0.000	<0.001*
48 hrs	65603.6	753.12	31781.6	558.0	0.000	<0.001*
72 hrs	75435.6	435.7	67536.2	733.3	0.000	<0.001*
96 hrs	306146.7	3397.2	413815.1	129588.0	10.000	0.002*
120 hrs	512754.2	3463.9	1640872.0	2451.78	0.000	<0.001*
240 hrs	858376.8	4631.2	3935379.8	5887753.6	0.000	<0.001*
264 hrs	1972109.9	9355.3	4628975.3	6954141.5	10.00	0.002*
288 hrs	2484429.0	5872.1	5549451.2	8316678.5	0.000	<0.001*
336 hrs	3563766.6	7623.4	3951686.8	1235806.7	10.000	0.002*
380 hrs	4801315.0	12413.0	5755508.8	6128.7	0.000	<0.001*

Mann-Whitney U test (significant for p<0.05*).

Table 4. Evaluation of surface hardness.

Hardness (Vickers hardness (VHN)	Group	Mean	SD	p-value
After treatment with test material	***Test***	362.78	26.67	0.07

Vickers hardness was measured using a 500-g load and a 15-s loading time (Micromet 2100, Buehler). Vickers hardness means (VHN) and standard deviations were analyzed statistically by Kruskal-Wallis non-parametric test at 5% significance level, the material showed no significant difference (p=0.07) between both treatment groups.

DISCUSSION

Peri-implantitis has been described as a site-specific condition or as an inflammatory bacterial driven destruction of the implant supporting tissues, in which microorganisms plays an important role in peri-implantitis (Mombelli *et al*., 1987). Effective decontamination of dental implant surfaces is one of the most difficult steps and, for this reason, several different treatments have been proposed in literature (Schwarz *et al*., 2011). However, some of these methods can damage the surface properties of implants or promote bacterial resistance (Norowski Jr *et al*., 2009).

Thus, antimicrobial photodynamic therapy (PDT) is a non-invasive mode of treatment and has been proposed as an adjuvant intervention for periodontitis. The antimicrobial effect of PDT is based on particular interaction of a specific dye that attaches to the surface of microorganism, leading to lethal changes in target bacteria. Due to this high absorption of radiation energy by the dye, this process ultimately leads to a functional disintegration of microorganisms. Several dyes, also referred to as photosensitizers, are firmly established in dentistry (Madi *et al*., 2018).

Antimicrobial photosensitizers such as porphyrins, phthalocyanines, and phenothiazines (e.g., methylene blue and toluidine blue) have been reported to penetrate into gram-positive and gram-negative bacteria. The positive charge seems to promote the binding of the photosensitizer to the gram-negative bacterial membrane, and leads to its localized damage, resulting in an increase in its permeability. Studies have already shown a significant reduction in bleeding values as bacterial counts of periodontal pathogens after adjuvant use of PDT with different photosensitizers, when compared with conservative treatment alone (Suchetha *et al*., 2017). Aluminum phthalocyanines have shown good inactivation of various microbial pathogens. The amphiphilic character of photosensitizer is an important property in photodynamic inactivation of microbes. This property may be enhanced by cationic charges, the number of these cationic charges, distribution of cationic charge, and the substituted group at lipophilicity of photosensitizer molecule (Ragas X *et al*., 2013).

Valle-Molinares *et al*. (2015) also investigated the effect of tetra-carboxy phthalocyanine against resistant strain microbes (*S. aureus*, *Klebsiella pneumoniae* and *E. coli)*. The results showed a higher inhibition of over 80% on all the tested strains of microbes. The effectiveness of this compound as antimicrobial may be attributed to inclusion of carboxyl group in phthalocyanine structure, which provides adhesion of this molecule on the surface of the microbes. Therefore, assessing bacterial count and surface hardness together by application of PDT with aluminum phthalocyanines on bacterial coated titanium discs may provide a clear insight into the effect of antimicrobial activity of PDT.

In a study in dogs, Shibli *et al*. (2003) investigated the effects of PDT on peri-implantitis and reported that PDT was able to reduce bacterial counts. *Prevotella sp.*, *Fusobacterium sp.* and *S. beta-haemolyticus* were not 100% destroyed in all samples, although complete elimination of those pathogens was achieved in some samples. Cai *et al*. (2019) incubated *S. aureus* biofilm on polished and sandblasted large-grit acid-etched (SLA) titanium surfaces for 48hrs, which were then randomly grouped for treatment protocols with phosphate-buffered saline, 0.2% chlorhexidine (CHX), 3% hydrogen peroxide (H_2O_2), PDT 0.2% CHX + PDT, and 3% H_2O_2 + PDT. Their results concluded that 0.2% CHX + PDT was more effective in eradicating *S. aureus* when compared with either treatment alone, as was 3% H_2O_2 + PDT. This is suggestive that PDT provides an added benefit.

A study by Zafar *et al*. (2016) reviewed in detail the photophysical and photochemical parameters of the aluminum phthalocyanine derivatives, as well as their *in vitro* and *in vivo* cytotoxic activities, diagnostic aspects and antimicrobial efficacy. They concluded that, taking into account the antimicrobial PDT, cationic derivatives show promising results against virus, bacteria and antibiotic resistant bacteria as well. In the present study, we compared the combination of mechanical debridement or PDT alone in eliminating *S. aureus*, *F. nucleatum* and *P. gingivalis* biofilm from different titanium surfaces. From the results of this *in vitro* study, single application of any disinfection modality has achieved significant bacterial reduction, compared to control group. On intragroup comparison, of both test and control groups for the three different microorganisms (*S. aureus, F. nucleatum, P. gingivalis*) at various time intervals, a highly significant amount of bacterial reduction was seen from 24hrs to 380hrs (p=0.0001).

Our results were in line with some previous studies (Salvi *et al*., 2008; Harris *et al*., 2006). In our study *S. aureus* was considered because it shows a specific affinity to titanium surfaces. The ability of *S. aureus* to adhere to extracellular matrix components and plasma proteins deposited on biomaterial surfaces, eventually leading to a biofilm formation, represents a critical step in the pathogenesis of implant-associated infections, which was reported to be sensitive to PDT treatment due to its relatively porous cytoplasmic membrane. In the mean bacterial count, there was a highly significant reduction in the bacteria load gradually at various time intervals from 24hrs to 380hrs.

The present study is in accordance with Labban *et al*. (2021), who evaluated the efficacy of indocyanine-green (ICG) mediated PDT through delivery of ICG solution at a concentration of 1 mg/mL at the bottom of the peri-implant pocket using a 1 mL syringe and 810nm diode laser. It was observed that reduction of both *P. gingivalis* and *T. denticola* was significantly higher when

dental implants were treated with ICG-PDT. Therefore, PDT with photosensitizer is an effective treatment modality for bacterial elimination in periodontal and peri-implant diseases. Their study also demonstrated significant reduction of bacterial colony count due to PDT.

Bassetti *et al*. (2014) compared adjunctive PDT and local drug delivery in treatment of initial peri-implantitis. After 1 year, counts of *P. gingivalis* and *T. forsythia* decreased significantly in both groups, suggesting adjunctive PDT might represent an alternative treatment modality in nonsurgical management of initial peri-implantitis.

Intergroup comparisons of *P. gingivalis* in test groups at various time intervals from 24hrs to 380hrs showed highly significant bacterial reduction. According to Albaker *et al*. (2018), antimicrobial PDT has advantages such as a lower risk of bacterial resistance, suppression of the oral microbiota at the application site, and absence of systemic harmful effects; in addition, this therapy does not cause damage at the site of application. The treatments were found to be beneficial, improving inflammation and the immunological status of tissues.

The results obtained in the present study are in accordance with several studies in the literature (Ragas *et al*., 2013; Harris *et al*., 2006; Bassetti *et al*., 2014; Albaker *et al*., 2018). The control group showed no reduction in bacterial count with mechanical debridement alone, while in the test group, when a combination of mechanical debridement along with PDT was used, there was reduction in bacterial count, in comparison with control group. Therefore, the use of dye was effective in achieving greater bacterial reduction, and this difference was statistically significant ($p<0.001$). This result proves the PDT had an added advantage over laser therapy, with its antimicrobial and subsequent anti-inflammatory properties.

On intergroup comparison, the mean values at different time intervals between 48hrs and 72hrs revealed no significant difference in test group ($p=0.940$, $p=0.199$, respectively). The above results are in accordance with the study by Marotti *et al*. (2013), in which they have used laser irradiation with dye application at different time intervals in two groups, to evaluate the effectiveness of the use of dye on the action of PDT. It can be concluded that shorter duration of dye in contact with implant surfaces shows impact on the number of bacterial reduction count.

A study by Chauhan *et al*. (2021) indicated that the increase in the hardness of the samples subjected to laser treatment depends on increase in the laser operating parameters, and it was noted that overly high value of the laser operating parameters was not able to effectively improve the microhardness of a surface. It has been shown that laser treatments resulted in no effect on surface hardness without affecting Ti biocompatibility. Thus, it is plausible to propose that combined application of PDT with photosensitizer suppresses *S. aureus*, *P. gingivalis* and *F. nucleatum* biofilm formation on Ti discs. This underlines combined application of AlPc dye with PDT should be regarded as an efficient adjunct therapy to mechanical therapy in peri-implantitis treatment.

Limitations

The present study has certain limitations that need to be contemplated to interpret the results. *T. forsythia* was not included in the study along with *P. gingivalis* and *F. nucleatum* because of the lack of availability of the bacteria. Thus, further studies with the effect on *T. forsythia* should be conducted. Titanium discs were polished, but the implants used in the clinic are subjected to various surface treatments, so the results of the study may differ in clinical practice. It can be inferred from the present study that there is reduction in the bacterial colony count with antimicrobial photodynamic therapy using a novel photosensitizer (AlPcOH) in test group. Antimicrobial PDT using the novel photosensitizer may enhance the potential benefits of mechanical treatment, and can be used as an adjunct to non-surgical therapy. However, clinical studies with long term follow up and comparison of PDT with other treatment protocols should be carried out, considering the limitations.

Conclusion

This present *in vitro* study concluded that PDT with hydroxyaluminium phthalocyanine (AlPcOH) dye was effective in eradicating *S. aureus*, *F. nucleatum* and *P. gingivalis* on Ti discs. This study concluded that the PDT with photosensitizer can be used as an adjunct in management of peri-implantitis.

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