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RESEARCH ARTICLE

EVALUATING ANTIMICROBIAL EFFICACY OF DIFFERENT TREATMENT MODALITIES ON DENTAL IMPLANT SURFACE: AN IN VITRO STUDY

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ABSTRACT

Background: Dental implants for replacing missing teeth has become standard practice in dentistry. Biological complications like peri-implantitis is common and involves biofilm-induced inflammation and bone loss around implants.

Material and Methods: Sixty standardized titanium discs (10 mm diameter, 2 mm thickness) were fabricated from grade 4 commercially pure titanium. All discs underwent sandblasting and acid etching to create a surface topography representative of contemporary dental implants. Surface characterization was performed using scanning electron microscopy (SEM) to ensure standardization across specimens. The discs were sterilized by gamma irradiation prior to biofilm formation.

Results: Er:YAG laser and photodynamic therapy achieved the highest bacterial reductions (94.3% and 92.1%).However, Er:YAG laser treatment also caused noticeable surface alterations, including melting and re-solidification patterns and smoothing of some surface irregularities.

Conclusion: Photodynamic therapy caused minimal surface alteration compared to other modalities. It may offer the best balance between antimicrobial efficacy and surface preservation in peri-implantitis management.

Keywords: Dental implants; peri-implantitis; biofilm; decontamination; photodynamic therapy; Er:YAG laser; chlorhexidine; air polishing

1. INTRODUCTION

The use of dental implants for replacing missing teeth has become standard practice in contemporary dentistry, with high long-term survival rates. However, the increased prevalence of implant-supported restorations has been accompanied by a rise in biological complications such as peri-implant mucositis and peri-implantitis. Peri-implantitis is an inflammatory condition affecting the tissues surrounding dental implants, characterized by inflammation of the peri-implant mucosa and progressive loss of supporting bone[1–4]. This pathological condition affects approximately 20% of patients with dental implants, posing significant challenges to long-term implant success and patient oral health[5].

The primary etiological factor for peri-implant diseases is bacterial biofilm accumulation on implant surfaces. The microbial composition associated with peri-implantitis resembles that of chronic periodontitis, with predominance of gram-negative anaerobic bacteria including *Porphyromonas gingivalis, Tannerella forsythia, Fusobacterium nucleatum, and Prevotella intermedia.* Unlike natural teeth, dental implants lack a periodontal ligament and cementum, resulting in different host responses to bacterial challenge. Additionally, the micro-roughened surfaces of modern implants, while beneficial for osseointegration, create an environment conducive to bacterial adhesion and biofilm formation[6,7].

Effective management of peri-implantitis necessitates thorough decontamination of implant surfaces to eliminate pathogenic microorganisms and create conditions favorable for re-osseointegration or soft tissue reattachment[2]. The complex topography of dental implants, characterized by threads, microgrooves, and varying surface treatments, presents significant challenges for conventional mechanical debridement alone[6,8]. Consequently, numerous adjunctive decontamination methods have been developed and investigated over the past decades.

Chemical decontamination approaches using antimicrobial agents such as chlorhexidine have been widely studied. Chlorhexidine (CHX), a cationic bisbiguanide, demonstrates broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria through disruption of bacterial cell membranes[1]. Its substantivity allows a prolonged antimicrobial effect after application. However, concerns regarding cytotoxicity to osteoblasts and potential alterations to implant surface properties have been raised[5].

Laser therapy has emerged as a promising decontamination approach, with various types of lasers investigated for peri-implantitis treatment. The Er:YAG laser, in particular, has shown efficacy in biofilm removal without causing significant thermal damage to surrounding tissues when used with appropriate parameters[9]. Its ability to access areas difficult to reach through mechanical means makes it an attractive option for implant surface decontamination[10–12].

Photodynamic therapy (PDT) combines a photosensitizing agent (typically a dye such as methylene blue) with light of a specific wavelength to generate reactive oxygen species that exert antimicrobial effects. Several studies have reported significant bacterial reduction on implant surfaces following PDT application without adverse effects on surface properties[12]. The non-invasive nature of PDT and the absence of bacterial resistance development are notable advantages of this approach.

Air polishing systems utilize pressurized air, water, and abrasive powders to mechanically remove biofilm from implant surfaces. Recently, low-abrasive powders such as glycine have been introduced to minimize damage to implant surfaces while maintaining cleaning efficacy. Studies have demonstrated that air polishing with glycine powder can effectively reduce bacterial load on various implant surfaces with minimal surface alterations.

Other emerging approaches include ozone therapy, which utilizes the strong oxidizing properties of ozone to eliminate microorganisms;[6] hydrogen peroxide photolysis, which generates hydroxyl radicals with potent antimicrobial activity;[13] and plasma of argon treatment, which has shown promise in implant surface decontamination while preserving favorable surface characteristics for cell adhesion[14].

Therefore, this study aimed to comparatively evaluate the antimicrobial efficacy of four commonly used decontamination methods like chlorhexidine irrigation, Er:YAG laser treatment, photodynamic therapy, and air polishing with glycine powder on biofilm-contaminated titanium implant surfaces. The null hypothesis was that there would be no significant differences in bacterial reduction among the tested decontamination methods. Additionally, we sought to assess the effects of these treatments on implant surface topography, as surface alterations may influence subsequent cellular responses and healing potential.

2. MATERIAL AND METHODS

This in vitro experimental study was designed to evaluate and compare the antimicrobial efficacy of different treatment modalities on biofilm-contaminated titanium implant surfaces.

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Sixty standardized titanium discs (10 mm diameter, 2 mm thickness) were fabricated from grade 4 commercially pure titanium. All discs underwent sandblasting and acid etching to create a surface topography representative of contemporary dental implants. Surface characterization was performed using scanning electron microscopy (SEM) to ensure standardization across specimens. The discs were sterilized by gamma irradiation before biofilm formation.

Biofilm Formation

A three-species biofilm model consisting of *Porphyromonas gingivalis* ATCC 33277, *Fusobacterium nucleatum* ATCC 25586, and *Streptococcus mitis* ATCC 49456 was developed to simulate peri-implantitis biofilm. This combination was selected based on their established role in peri-implant diseases. Bacterial strains were cultivated in appropriate growth media under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) at 37°C for 48 hours. Bacterial suspensions were adjusted to 1×10^8 CFU/mL using spectrophotometric analysis.

Sterile titanium discs were placed in 24-well plates and incubated with 2 mL of the bacterial suspension for 72 hours at 37°C under anaerobic conditions to allow biofilm formation. Culture media were replaced every 24 hours. After incubation, discs were gently rinsed twice with phosphate-buffered saline (PBS) to remove non-adherent bacteria while preserving the biofilm structure.

Experimental Groups

The biofilm-contaminated titanium discs were randomly allocated to five experimental groups (n=12 per group):

1. Group CHX: Irrigation with 0.2% chlorhexidine digluconate solution for 60 seconds
2. Group ER: Er:YAG laser irradiation (2940 nm, 100 mJ/pulse, 10 Hz, 60 seconds, non-contact mode, distance 0.5 mm)
3. Group PDT: Photodynamic therapy using methylene blue (0.01%) as a photosensitizer for 60 seconds, followed by irradiation with a diode laser (660 nm, 100 mW/cm², 60 seconds)
4. Group AIR: Air polishing with glycine powder (mean particle size 25 μm, pressure 2 bar, distance 5 mm, 10 seconds)
5. Group CON: Irrigation with sterile saline solution for 60 seconds (control)

All treatments were performed by a single calibrated operator to minimize technique variability. After treatment, all specimens were rinsed with sterile PBS to remove residual agents or debris.

Microbiological Analysis

After decontamination procedures, six randomly selected specimens from each group were transferred to sterile tubes containing 10 mL of reduced transport fluid. Biofilms were dislodged by vortexing for 30 seconds, followed by ultrasonic dispersion for 60 seconds. Serial tenfold dilutions were prepared and plated on appropriate selective media. After anaerobic incubation for 7 days, colony-forming units (CFU) were counted by a blinded examiner. Bacterial reduction percentages were calculated relative to the control group.

Surface Topography Analysis

The remaining six specimens from each group were fixed in 2.5% glutaraldehyde, dehydrated in a graded ethanol series, and sputter-coated with gold for SEM analysis. Surface characteristics, including roughness, presence of debris, and surface alterations, were evaluated at various magnifications (500×, 1000×, and 3000×) by a trained examiner blinded to the treatment groups.

Statistical Analysis

Sample size calculation determined that 12 specimens per group would provide 80% power to detect a difference of 15% in bacterial reduction with $\alpha=0.05$. Statistical analysis was performed using SPSS software version 26.0. The Shapiro-Wilk test was used to assess data normality. Differences in bacterial reduction among groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test. A p-value <0.05 was considered statistically significant.

3. RESULTS

The quantitative microbiological analysis revealed significant differences in bacterial reduction among the five experimental groups ($p<0.001$). **Table 1** presents the mean bacterial counts (CFU/mL) and bacterial reduction percentages for each treatment modality compared to the control group.

Table 1. Mean bacterial counts and bacterial reduction percentages across treatment groups

Treatment Group	Mean Bacterial Count (CFU/mL)	Log_{10} Reduction	Bacterial Reduction (%)
Control (Saline)	$2.7 \times 10^8 \pm 3.4 \times 10^7$	-	-
Chlorhexidine 0.2%	$4.6 \times 10^7 \pm 1.8 \times 10^7$	0.77	82.7
Er:YAG Laser	$1.5 \times 10^7 \pm 0.9 \times 10^7$	1.26	94.3
Photodynamic Therapy	$2.1 \times 10^7 \pm 1.3 \times 10^7$	1.11	92.1
Air Polishing	$3.1 \times 10^7 \pm 1.5 \times 10^7$	0.94	88.5

All decontamination methods demonstrated significantly higher bacterial reduction compared to the saline control ($p<0.001$). The Er:YAG laser treatment showed the highest bacterial reduction (94.3%), followed by photodynamic therapy (92.1%), air polishing (88.5%), and chlorhexidine irrigation (82.7%). Post-hoc analysis revealed that Er:YAG laser and photodynamic therapy groups exhibited significantly higher bacterial reduction compared to chlorhexidine ($p=0.023$ and $p=0.036$, respectively). No statistically significant difference was observed between Er:YAG laser and photodynamic therapy groups ($p=0.627$).

Analysis of individual bacterial species revealed varying susceptibilities to the different decontamination methods, as shown in **Table 2**.

Table 2. Bacterial reduction percentages by species across treatment groups

Treatment Group	*P. gingivalis* Reduction (%)	*F. nucleatum* Reduction (%)	*S. mitis* Reduction (%)
Chlorhexidine 0.2%	86.4 ± 5.2	84.1 ± 4.9	77.5 ± 6.3
Er:YAG Laser	95.8 ± 2.1	93.7 ± 3.5	93.4 ± 3.8
Photodynamic Therapy	94.3 ± 3.4	92.5 ± 4.2	89.6 ± 5.1
Air Polishing	90.2 ± 4.6	87.9 ± 5.3	87.5 ± 5.7
Control (Saline)	25.3 ± 7.2	32.7 ± 8.1	38.4 ± 7.9

P. gingivalis showed the highest susceptibility to all decontamination methods, particularly to Er:YAG laser treatment (95.8% reduction). *S. mitis* demonstrated relatively greater resistance to chlorhexidine (77.5% reduction) compared to other treatment modalities. The differences in bacterial reduction among species were statistically significant for chlorhexidine ($p=0.018$) but not for Er:YAG laser ($p=0.072$), photodynamic therapy ($p=0.086$), or air polishing ($p=0.111$).

SEM analysis revealed varying degrees of surface alterations across the experimental groups. The untreated control specimens showed dense biofilm covering the titanium surface, with characteristic bacterial morphologies visible. Chlorhexidine-treated specimens demonstrated significant biofilm reduction with minimal alterations to the underlying surface topography. Some residual bacteria and organic debris were observed in surface irregularities. Photodynamic therapy resulted in substantial biofilm elimination while preserving the original surface characteristics of the titanium discs.

Er:YAG laser treatment achieved the most substantial biofilm removal but caused noticeable surface alterations, including melting and resolidification patterns and smoothing of some surface irregularities. Air polishing with glycine powder effectively removed biofilm with moderate surface changes, primarily characterized by rounding of sharp edges and slight ablation of surface peaks.

Qualitative assessment of surface alterations on a scale of 0 (no alterations) to 3 (severe alterations) yielded mean scores of 0.5 ± 0.3 for chlorhexidine, 1.9 ± 0.5 for Er:YAG laser, 0.4 ± 0.2 for photodynamic therapy, and 1.2 ± 0.4 for air polishing.

4. DISCUSSION

The superior antimicrobial efficacy of Er:YAG laser observed in our study (94.3% bacterial reduction) aligns with findings from previous investigations. Giannelli et al. reported that Er:YAG laser effectively eliminated bacteria from titanium surfaces with minimal thermal damage when appropriate parameters were used[9]. Similarly, Schwarz F et al. demonstrated that Er:YAG laser irradiation at low energy settings achieved significant biofilm removal without causing detrimental thermal effects on implant surfaces[5]. The high efficacy of Er:YAG laser can be attributed to its specific wavelength (2940 nm), which is strongly absorbed by water and hydroxyapatite, resulting in photoacoustic and photomechanical effects that disrupt biofilm without substantial heat generation. However, our SEM analysis revealed noticeable surface alterations following Er:YAG laser treatment, suggesting that while antimicrobial efficacy is excellent, potential changes to surface properties must be considered.

Photodynamic therapy demonstrated comparable antimicrobial efficacy (92.1% bacterial reduction) to Er:YAG laser while causing minimal surface alterations. These findings corroborate those of Hayek et al., who reported that PDT using methylene blue and a low-power laser effectively reduced bacteria on implant surfaces without affecting surface characteristics[2,15]. The mechanism of PDT involves the generation of reactive oxygen species, particularly singlet oxygen, which causes oxidative damage to bacterial cell walls, membranes, and DNA. Importantly, PDT does not induce bacterial resistance, unlike many conventional antimicrobial agents. Our SEM analysis confirmed that PDT preserved the original surface topography of titanium specimens, suggesting it may offer an optimal balance between antimicrobial efficacy and surface preservation.

Air polishing with glycine powder demonstrated good antimicrobial efficacy (88.5% bacterial reduction) with moderate surface alterations. These results are consistent with those reported by Schwarz et al., who found that air polishing with glycine powder effectively removed biofilm from various implant surfaces with minimal abrasive effects[16]. The less aggressive nature of glycine particles compared to conventional air polishing powders (e.g., sodium bicarbonate) contributes to the preservation of surface integrity while maintaining cleaning efficacy. Nevertheless, our SEM observations of some surface modifications suggest caution when using this technique, particularly on highly textured implant surfaces.

Chlorhexidine irrigation, while significantly reducing bacterial load (82.7%), showed the lowest antimicrobial efficacy among the tested modalities. This finding aligns with several previous studies that have questioned the effectiveness of chlorhexidine as a standalone treatment for implant surface decontamination. Kotsakis et al. reported that chlorhexidine irrigation achieved only partial biofilm removal, particularly in areas with complex surface topography[16]. Additionally, concerns have been raised regarding the potential cytotoxic effects of chlorhexidine on osteoblasts and its impact on re-osseointegration. Kotsakis et al. demonstrated that chlorhexidine, even at low concentrations, exhibited cytotoxicity to osteoblasts and inhibited cell migration[16–20]. These findings suggest that while chlorhexidine may be beneficial as an adjunct to mechanical debridement, alternative decontamination approaches might be more suitable for the comprehensive treatment of peri-implantitis.

The differential susceptibility of bacterial species to decontamination methods observed in our study has important clinical implications. *P. gingivalis*, a key periodontal pathogen strongly associated with peri-implantitis, showed high susceptibility to all tested methods, particularly Er:YAG laser and photodynamic therapy. In contrast, *S. mitis*, representing early colonizers in biofilm formation, demonstrated greater resistance to chlorhexidine. These findings support the notion that multispecies biofilms may require a combination of approaches for effective decontamination, targeting both early and late colonizers in the biofilm structure.

Surface topography plays a crucial role in subsequent biological responses following implant decontamination. While optimal roughness promotes re-osseointegration, excessive smoothing or surface damage may impair cellular attachment and healing. Our SEM analysis revealed that Er:YAG laser treatment, despite its excellent antimicrobial properties, caused the most pronounced surface alterations. This observation aligns with findings by Schwarz et al., who reported that Er:YAG laser irradiation at certain parameters could result in surface melting and crack formation[5]. In contrast, photodynamic therapy preserved surface characteristics while achieving comparable bacterial reduction, suggesting it may be preferable from a surface integrity perspective.

Several limitations of this study should be acknowledged. First, the in vitro nature of our investigation may not fully reflect the complex in vivo environment of peri-implantitis lesions, which involves host immune responses, varying oxygen tensions, and gingival crevicular fluid components. Second, our biofilm model, while incorporating key peri-implantitis-associated pathogens, represents a simplified version of the complex multispecies biofilms encountered clinically. Third, we evaluated only immediate antimicrobial effects; long-term

outcomes such as biofilm regrowth and surface biocompatibility require further investigation. Finally, the flat titanium discs used in our study do not replicate the complex three-dimensional geometry of dental implants, which may influence treatment efficacy, particularly in areas with limited accessibility[21,22].

5. CONCLUSION

Within the limitations of this in vitro study, Er:YAG laser treatment and photodynamic therapy demonstrated superior antimicrobial efficacy on biofilm-contaminated titanium surfaces compared to air polishing and chlorhexidine irrigation. However, Er:YAG laser caused more pronounced surface alterations, while photodynamic therapy preserved surface integrity while achieving comparable bacterial reduction. These findings suggest that photodynamic therapy may offer an optimal balance between antimicrobial efficacy and preservation of implant surface properties for the management of peri-implantitis. Clinical studies are needed to validate these findings and establish evidence-based protocols for implant surface decontamination in peri-implantitis treatment.

DECLARATIONS

Ethical approval and consent to participate

Not Applicable

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interest

The authors declare that there are no competing interests.

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