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Original Article

Comparison of antimicrobial effect of several decontaminating methods on contaminated Titanium discs

Reza Birang[1], Kourosh Shakerian[2], Ehsan Birang[3], Tahmineh Narimani[4], Narges Naghsh[5], Reza Fekrazad[6]

[1]Department of Periodontology, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, [4]Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, [5]Department of Periodontology, Dental Implants Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, [2]Department of Periodontology, School of Dentistry, Shahrekord University of Medical Sciences, Shahrekord, [3]Periodontist, School of Dentistry, International Campus, Iran University of Medical Sciences, Tehran, [6]Department of Periodontology, Dental Faculty-Laser Research Center in Medical Sciences, AJA University of Medical Sciences, Tehran, Iran

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Address for correspondence: Dr. Kourosh Shakerian, Department of Periodontology, Dental School, Shahrekord University of Medical Sciences, Shahrekord, Iran. E-mail: kourosh_shk@yahoo.com

ABSTRACT

Background: Decontaminating the implant surface, exposed to bacterial biofilm, is a concern in the treatment of peri-implant inflammatory disease. The aim of this study was to compare the effect of several methods on reduction of the bacterial load, colonized on the surfaces of titanium discs.
Materials and Methods: In this *in vivo* study, seven titanium discs with Sandblasted, Large-grit, acid-etched (SLA) surface were placed in the mouth of each of ten patients with chronic periodontitis by an intra-oral maxillary splint for 24 h. In each patient, the contaminated discs, except for the negative control ones, were randomly treated by one of the six antiseptic methods including sterile normal saline, plastic curette, air polisher, hydrogen peroxide, 980 nm diode laser, and Er-YAG laser. A spectrophotometer was used to measure Optical Density (OD) in case of aerobic microorganisms. Colony-Forming Units (CFUs) were used for anaerobic bacteria. Data were analyzed through Kruskal–Wallis and Mann–Whitney Tests at a significance level of α =0.05 by SPSS software.
Results: Statistical analysis showed a significant decrease in OD of aerobic bacteria among the seven groups during a 0–24 h time interval ($P < 0.001$). Furthermore, these tests showed a significant difference in the CFU ($P < 0.001$) for anaerobic bacteria after 48 h.
Conclusion: The results of this study showed that all of the adopted methods significantly reduced microbial colonies on the surfaces of titanium discs with SLA surface. Er:YAG laser and normal saline had the highest and the lowest effects, respectively.

Key Words: Decontamination, dental implants, laser

INTRODUCTION

The oral cavity has the potential to harbor at least 600 different bacterial species, and surfaces of the teeth can have as many as a billion bacteria in their attached bacterial plaques.[1] Biofilms are ubiquitous; they form on virtually all surfaces, immersed in natural aqueous environments including dental implants.[2] Periodontal disease is an infection of gums, that if not treated, can even lead to tooth loss. Dental implants are a broadly accepted and greatly predictable management modality in replacing natural teeth.[3]

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According to previous studies, oral microflora is a major factor in the development of peri-implant diseases and implants failure.[4,5] Therefore, the removal of bacterial biofilm from dental implant surface is the most important step in the control and treatment of peri-implant diseases.[6] However, the most effective method for decontamination of implant surface is still challenging.[7] So far, many mechanical (various types of plastic and titanium curettes, titanium brush, air-powder abrasive (APA) system and piezoelectric ultrasonic scaler), chemical (such as chlorhexidine [CHX], hydrogen peroxide [HP], and citric acid), and irradiating methods (including CO2, Diode, Nd: YAG and Erbium family (i.e. Er: YAG and Er, Cr: YSGG)) have been introduced to decontaminate implants surfaces.[7-12] Among irradiating methods, it has been proved that controlled irradiation of Diode and Er: YAG lasers, because of their poor absorption in titanium, can eliminate bacteria from implant surface without any increased heat in the implant body, and or superficial changes.[10,11,13] In addition, it has been reported that Er: YAG Laser could be used without mechanical means to remove bacterial biofilm whereas Diode and CO_2 lasers should be combined with mechanical methods.[11,14,15] Nevertheless, in an *in vitro* study, Schwarz *et al*. reported that 980 nm diode laser irradiation with a 3-watt power and CW mode fully removed the bacterial load from implant surface without mechanical methods, while irradiation with a 2.5-watt power was just able to partially reduce the bacterial number.[12] Despite the effectiveness of a variety of mechanical methods for the decontamination of dental implant surface, the majority of these ways may change the microstructure of implant surface and compromise the biocompatibility.[16-20]

Moreover, some chemical agents have been used to decontaminate implant surface. The most common agent is CHX which is widely used to treat peri-implantitis.[5] Currently, one of the chemicals, reported to have a positive effect on implant surface decontamination without any adverse effect, is HP.[21-23] However, currently, there is no standard and definite method to eliminate bacteria completely, to decontaminate the implant surface, and to treat peri-implant diseases.[24] Therefore, the aim of this study was to investigate the effects of six antiseptic methods including sterile normal saline, plastic curette, air polisher, HP, 980 nm diode laser, and Er: YAG laser on reducing the bacterial load on Titanium discs with sandblasted, large-grit, acid-etched (SLA) surfaces.

MATERIALS AND METHODS

In this *in vivo* study with ethical code of IR. MUI. Research. REC.1397.318, ten patients with chronic periodontitis (6 males and 4 females) with a mean age of 47.2 years referring to the department of periodontics, Isfahan University of Medical Sciences, Isfahan, Iran, were selected through a convenient nonrandomized sampling method. The informed consent forms were signed by the patients before entering the study. Inclusion criteria were having mild chronic periodontitis, based on clinical and radiographic examinations (attachment loss = 1–2 mm and bone loss = <30%),[25] no use of antibiotics and mouthwashes in the last two weeks prior to the study, and being a nonsmoker. In this study, seven titanium discs (5.3 mm in diameter and 1.5 mm in thickness) (Snucone Co., Daegu, Korea) with roughed surface of SLA (sandblasted with large grits and acid-etched on the surface of dental implant), fixed on an acrylic splint, were used for each patient.

Contamination stage

In order to contaminate titanium discs, an intra-oral maxillary splint was prepared ultrasonically, cleaned in 3% sodium hypochlorite, and then, was washed with distilled water.[19,21] Seven titanium discs, which had been sterilized by autoclaving at 121 ºC for 15 min,[21] were fixed bilaterally on the buccal surface of splint with sticky wax [Figure 1]. Patients (n = 10) were asked to wear the splints including discs, keep it in their mouth for 24 h and place it inside phosphate-buffered saline solution only during eating. In addition, they were asked to avoid brushing and cleaning the teeth for 24 h and clean their mouth only with tap water.[19,20]

Decontamination stage

The splints were removed from patients' mouth after 24 h and smoothly washed with sterile normal saline to remove debris and saliva. Then, the titanium discs of each host were categorized randomly through blinded method in 1 control (negative) and 6 experimental groups [Table 1]. The specimens of each group, except negative control ones, were decontaminated by one of the following six antiseptic methods:

Negative control group

In this group, the discs were not decontaminated.

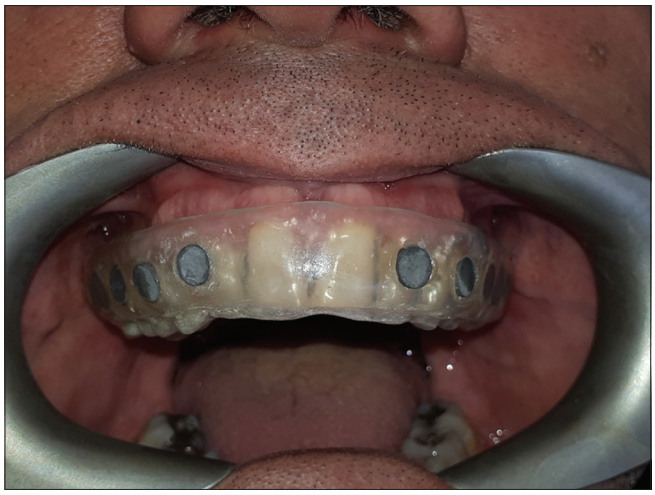

Figure 1: Intra-oral maxillary splint with titanium discs placed in the mouth.

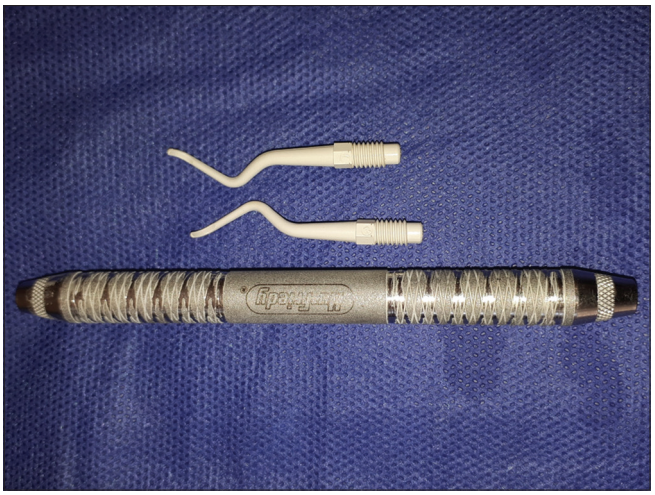

Figure 2: Plastic curette.

Er:YAG laser-treated group

In this group, an Er: YAG laser device (Fotona, Fidelis plus, Ljubljana, Slovenia) with a wavelength of 2940 nm was used. Laser parameters were set at 100 mJ/pulse (15.7J/cm^2), 10 Hz, and the pulse width of 250–300 µs.[11,18] The laser beam was guided onto the disc surface at a distance of 0.5–1 mm and 90° angle with up-and-down and side-to-side overlapping motions. The laser was radiated on the disc surface with the optical handpiece model RO7, and a tip of 900 µm in diameter, and water irrigation at 5 ml/min. The irradiation time was considered 1 min.[15,24]

Normal saline-treated group

The surface of the disc was cleaned by a cotton pellet, which was impregnated with normal saline through burnishing motion for 1 min.

Plastic curette treated group

The disc surface debridement was conducted with a plastic curette (Implacare™, Hu-Friedy, Chicago, IL, USA) at a 70° angle to the disc surface for 1 min [Figure 2].

Air-powder abrasive treated group

An air polisher device (NSK, Nakanishi, Japan), containing glycine powder with particle size range of 20–60 µm, was applied at a distance of 5 mm from the disc and an angle of 90° to the surface for 1 min. The power settings were 4 bar static pressure and 60 ml water/min.

Hydrogen peroxide + plastic curette treated group

After mechanical debridement by plastic curette for 1 min, disc surface decontamination was followed by a cotton pellet, impregnated with HP 3% (Merck CO, Darmstadt Germany) through burnishing motion for 1 min.

Table 1: Decontamination methods in different groups

Groups	Decontamination method
Group 1 (negative control)	Not decontaminated
Group 2 (positive control)	Er: YAG laser
Group 3	Normal saline
Group 4	Plastic curette
Group 5	Air- powder abrasive
Group 6	HP+plastic curette
Group 7	980 nm diode laser+plastic curette

Er: YAG: Erbium yttrium aluminium garnet

980 nm diode laser + plastic curette treated group

After mechanical debridement by a plastic curette for 1 min, the disc surface was decontaminated with a 980 nm diode laser (Fox A. R. C. Laser, Gmbh Germany) with the power of 1W and continuous wave (CW) with a 90° angle and a distance of 0.5–1 mm, and with an up-and-down and side-to-side overlapping motions for 1 min.[21,27]

In all of the studied groups, at the end of the decontamination stage, the disc surfaces were washed with 5 cc sterile normal saline,[28] and then, the bacterial sampling was done. Meanwhile, all of stages were done by an expert researcher.

Laboratory stage

The culture medium for anaerobic samples included plates containing Colombia-Agar and Hemin and Vit K and Blood 5%, on which the samples were transferred by sterilized swabs from the surface of titanium discs. In order to create anaerobic conditions,

the plates were placed in an anaerobic jar immediately after sampling. The air inside the jars was evacuated with a gas pack and a palladium catalyst. Anaerobic bacterial colonies in each plate were counted after 48 h by Colony-Forming Units (CFUs).

Aerobic samples were prepared from the disc surface by swabs, and then, placed into a test tube containing TSB (Triptocase Soy Borth). The tubes were then sent to the laboratory to determine optical density (OD) in a spectrophotometer (PG Instrument Ltd England). OD samples were determined at zero (immediately after transfer to laboratory) and a 24-h interval with wavelength of 620 nm. Aerobic samples were kept in an incubator at 37°C in time interval of 0 and 24 h.[29,30] In this stage, the microbiologist operator was blind to all of the study groups.

Data analysis stage

All analyses were conducted at the significance level of α =0.05 in SPSS software (version 22, IBM Corporation, Armonk, NY). To compare the normal distribution of the data, Kolmogorov–Smirnov test was conducted for aerobic (OD variation) and anaerobic (CFUs) samples. To Consider the lack of normality, Kruskal–Wallis, and Mann–Whitney tests were used with regard to the Dunn test and Bon Ferroni correction for the significance level.

RESULTS

Regarding the failure in assumptions of One-way ANOVA test, Kruskal–Wallis test was conducted to compare the data, obtained from the 7 groups of aerobic bacteria [Figure 3]. This test showed a significant difference between times of zero and 24 h ($P < 0.001$) in the seven groups, in terms of OD variation index (OD variation). In addition, to investigate the effect of the methods on anaerobic bacteria after 48 h, Kruskal–Wallis test showed a significant difference in the CFUs index [Figure 4] ($P < 0.001$). In order to compare groups pair by pair, Man–Whitney test was used [Table 2]. According to the results, Er: YAG laser had the most effect on reduction of the bacterial count (both aerobic and anaerobic) adhering to the titanium disc ($P < 0.001$). In contrast, the pair comparison of the groups in the present study showed that normal saline and plastic curette had the least effect, respectively ($P = 0.068$).

DISCUSSION

Bacterial contamination of implant surface is one of the major etiologic factors in pathogenesis of peri-implant diseases. It has been suggested that the effective therapeutic approach should be based on removing the bacterial plaques adhering to the implant surface as they prevent adhesion of the osteoblasts to the implant surface and re-osseointegration.[31,32]

In this *in vivo* study, the effects of several methods of decontamination on the reduction of the bacteria adhering to the surface of titanium discs were evaluated. The results of this study in both aerobic and anaerobic bacteria showed that Er: YAG and 980 nm diode lasers were the most effective methods on reducing bacterial load, respectively. The determination of Er: YAG laser as a positive control and its comparison with other methods was according to various studies reporting its positive

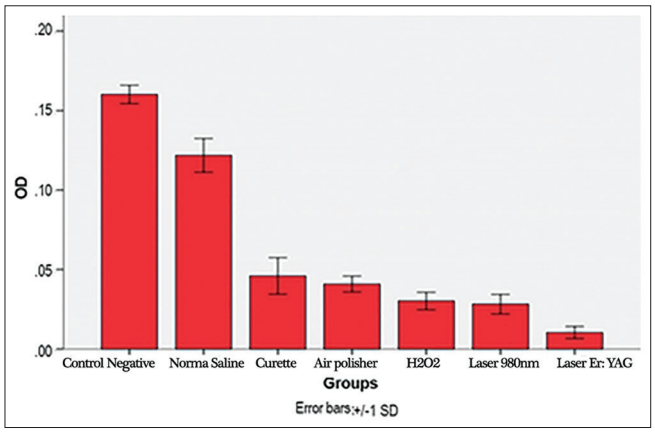

Figure 3: Comparison of the effects of decontamination methods on the number of aerobic bacteria. OD: Optical density.

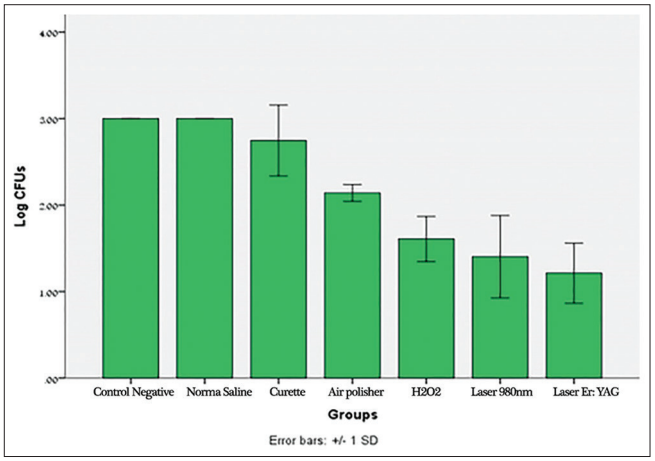

Figure 4: Comparison of the effects of decontamination methods on the number of anaerobic bacteria. CFUs: Colony-forming units.

Table 2: Two by two comparison of the effect of different decontamination methods on aerobic and anaerobic bacteria

Studied groups	OD variations during 24 h (aerobic bacteria) (*P*)	CFUs within 48 h (anaerobic bacteria) (*P*)
Normal saline - N-CO	<0.001	1.00
Curette - N-CO	<0.001	0.068
Air polisher - N-CO	<0.001	<0.001
H2O2 - N-CO	0.001	<0.001
980 nm diode laser - N-CO	<0.001	<0.001
Er: YAG - N-CO	<0.001	<0.001
Normal saline - curette	<0.001	0.068
Normal saline - air polisher	<0.001	<0.001
Normal saline - H2O2	<0.001	<0.001
Normal saline - 980 nm diode laser	<0.001	<0.001
Normal saline - Er: YAG	<0.001	<0.001
Curette - air polisher	0.172	0.006
Curette - H2O2	0.003	<0.001
Curette - 980 nm diode laser	0.002	<0.001
Curette - Er: YAG	<0.001	<0.001
Air polisher - H2O2	<0.001	<0.001
Air polisher - 980 nm diode laser	<0.001	<0.001
Air polisher - Er: YAG	<0.001	<0.001
H2O2-980 nm diode laser	0.303	0.087
H2O2 - Er: YAG	<0.001	0.007
980 nm diode laser - Er: YAG	<0.001	0.184

Er: YAG: Erbium yttrium aluminium garnet; OD: Optical density; CFU: Colony-forming units; N-CO: Negative control group

effects and acceptable performance.[11,33-36] In addition, in our previous study, Er: YAG laser was considered as a positive control, and the results showed that Er: YAG laser, compared to other methods including 810 nm diode laser, plastic curette, CHX (0.12%) and photodynamic therapy (660 nm diode laser + tolonium chloride), was the most effective method in both groups of aerobic and anaerobic bacteria.[34]

In addition, it has been reported that Er: YAG laser has the ability to remove biofilms from smooth and rough surfaces of titanium implants due to photomechanical ablation and seems to be a promising way for clinical use to remove bacterial plaque and calcified deposits to treat pri-implantitis.[37,38] However, it has been reported that Er: YAG laser with setting parameters of 100 mJ/pulse 10 Hz was unable to fully remove dental biofilms from titanium rough surfaces.[27] Also, Kreisler *et al*. reported that the Er: YAG laser, with the aforementioned parameters, failed to provide the previous biocompatibility of the SLA titanium surfaces sufficiently for the adhesion and growth of osteoblast cells (SAOS2 Osteoblasts).[11]

Recently, Aoki *et al*. used Er: YAG laser irradiation with two different parameters of 80 mj/pulse (20.3 j/cm^2), 12 Hz, and 150 mj/pulse (38.2 j/cm^2), 12 Hz for 60 s on the titanium discs, contaminated with subgingival plaque, obtained from peri-implantitis patients. They reported that 150 mj/pulse irradiation completely eliminated both the bacterial plaque and also titanium oxide layer (TiO_2) from the surface of titanium discs. Based on the SEM images, the surface of discs in this group had no signs of melting or other heat-induced deformation. While in 80 mj (20.3 j/cm^2) irradiation group, some plaque residue (about 32.2%) and a large amount of TiO_2 were observed. They also reported that the surfaces of the discs, treated by Er: YAG laser irradiation with 150 mj parameter, provided favorable conditions for the growth of osteoblasts (SAOS2 osteoblasts), but in 80 mj group, a large amount of remaining plaque prevented the adhesion and growth of osteoblast cells.[39]

Therefore, by comparing the findings of the present study with the results of some previous ones,[11,18,28,38] the setting parameters, adopted in the study of Giannelli *et al*. (150 mj/pulse, 12 Hz) for Er: YAG laser irradiation in full decontamination of titanium implants with a rough surface, might be more appropriate than those, used in the present study (100 mj/pulse, 10 Hz). Hence, Er: YAG laser could be more reliable to decontaminate dental implants surfaces. However, the high cost of this system and the need for a climatic equipment may make lighter and cheaper lasers such as diode more demanding.

According to the results of this study, the 980 nm diode laser showed the highest efficacy after Er: YAG, compared to other methods. Unlike the Er: YAG laser, diode laser irradiation on implant surfaces alone may not be able to remove all the biofilm adhering to the surface,[39] so the combination of this laser and mechanical methods is recommended.[14,40] The results of many studies indicate that diode laser irradiation on different surfaces of implant causes no superficial changes.[12,13,41] However, the increased temperature in diode laser irradiation spreads to the surrounding bone.[42-45] Increased temperature for more than 10° can damage bone tissue.[46] Therefore, controlled setting parameters and limitation of irradiation time are important factors in using diode lasers to decontaminate implant surface.[11,13,45] Neglecting these safety considerations can endanger the survival of the implant.[46]

In contrast, the pair comparison of the groups in the present study showed that normal saline and plastic curette had the least effects respectively. Eriksson *et al*. investigated the efficacy of normal saline on the treatment of peri-implantitis. In their study, the titanium contaminated surface was cleaned by sterile saline normal. Their results showed a significant decrease in lipopolysaccharide levels, compared to untreated implants.[47] In a study on animals, Zablotsky *et al*. claimed that there was no difference between applying APA method, citric acid, CHX, and saline in different combinations of implants surfaces. However, no positive result can be concluded in using saline alone from their study.[48]

Persson *et al*. obtained no positive outcomes using normal saline in the treatment of lesions surrounding implants.[49] In a comprehensive review, Suarez *et al*. found that evaluation of the value of normal saline in the treatment of the contaminated surfaces of implants is very difficult because it was used with other decontaminants.[8] Also according to the findings of the present study, decontaminating by plastic curette alone showed poor results. These results are in agreement with previous reports.[19,39,50]

The results of group comparison in the present study showed that the HP 3% had approximately the same effect of the 980 nm diode laser. The effect of this agent against bacteria is due to oxidizing action.[22] Hinrichs *et al*. compared HP 3% with several antibacterial agents on oral biofilms. They reported that HP with CHX, sodium hypochlorite and Listerine had a significant bactericidal effect against the bacteria adhering to titanium surface although none of these materials could completely eliminate the bacteria.[26] These findings are consistent with the results of the present study. Louropoulou *et al*. compared HP 3% with several acidic and nonacidic solutions including 0.012% CHX and 15% HP on titanium discs and titanium alloy through two methods of immersing and rubbing. Their results showed that the use of HP 3% with both methods caused no changes on the surface of the discs, while other solutions showed overt changes on the discs.[51] The results of this study showed the importance of the type and concentration of the solution.

APA system is a method, initially used to remove the stains from the surface of enamel, but later, it was used on implants.[8] The first time, Wheelis *et al*. investigated the effect of APA on the surface of titanium implants in an *in vitro* study. Their results showed that 100% of the bacteria were removed from the discs, exposed to APA.[51] Kreisler *et al*. also compared Er: YAG laser irradiation and APA system (with sodium bicarbonate) on the surface of the titanium discs, contaminated with bacterium *Porphyromonas gingivalis*. They concluded that in the APA group, despite the occurrence of visible changes on the disc surface, the greatest proliferation of fibroblastic cells occurred. Shou *et al*. reported that amino acid glycine powder, in spite of a less abrasive power, compared to sodium bicarbonate powder, had the same efficiency of bacterial biofilm removal as sodium bicarbonate.[50] These findings indicate the appropriate potentiality of this method to remove the cytotoxic bacterial components from the implant surface.[10] Meanwhile, our results are not consistent with the results of the above studies on the removal of bacteria from implant surface. One of the main factors of this mismatch in the results can be the type of abrasive powder. In addition, factors such as working time, the difference in surface roughness of the implants, distance of abrasive device tip from implant surface while using it, and the type of bacterial contamination may be the reasons for controversial results. In general, according to available data, APA seems to be an effective way in removing bacterial biofilms from smooth and rough dental implant surfaces without marked changes.[51] However, an absolute conclusion about the efficacy of these methods requires further studies, especially clinically controlled studies to understand their effectiveness.

CONCLUSION

Within the limits of this study, between studied groups, Er: YAG laser was the most effective method for reduction of the bacteria adhering to the titanium disc surface, contaminated with oral biofilm. Although 980 nm laser and HP had relatively good effects, normal saline, plastic curette, and APA methods showed poor effects.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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