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ORIGINAL ARTICLE

Comparison of decontamination efficacy of two electrolyte cleaning methods to diode laser, plasma, and air-abrasive devices

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Abstract

Objective To compare the in vitro decontamination efficacy of two electrolytic cleaning methods to diode laser, plasma, and air-abrasive devices.

Material and methods Sixty sandblasted large-grit acid-etched (SLA) implants were incubated with 2 ml of human saliva and Tryptic Soy Broth solution under continuous shaking for 14 days. Implants were then randomly assigned to one untreated control group ($n=10$) and 5 different decontamination modalities: air-abrasive powder ($n=10$), diode laser ($n=10$), plasma cleaning ($n=10$), and two electrolytic test protocols using either potassium iodide (KI) ($n=10$) or sodium formate ($CHNaO_2$) ($n=10$) solution. Implants were stained for dead and alive bacteria in two standardized measurement areas, observed at fluorescent microscope, and analyzed for color intensity.

Results All disinfecting treatment modalities significantly reduced the stained area compared to the untreated control group for both measurement areas ($p<0.001$). Among test interventions, electrolytic KI and $CHNaO_2$ treatments were equally effective, and each one significantly reduced the stained area compared to any other treatment modality ($p<0.001$). Efficacy of electrolytic protocols was not affected by the angulation of examined surfaces [surface angulation 0° vs. 60° (staining %): electrolytic cleaning-KI 0.03 ± 0.04 vs. 0.09 ± 0.10; electrolytic cleaning-CHNaO2 0.01 ± 0.01 vs. 0.06 ± 0.08; ($p>0.05$)], while air abrasion [surface angulation 0° vs. 60° (staining %): 2.66 ± 0.83 vs. 42.12 ± 3.46 ($p<0.001$)] and plasma cleaning [surface angulation 0° vs. 60° (staining %): 33.25 ± 3.01 vs. 39.16 ± 3.15 ($p<0.001$)] were.

Conclusions Within the limitations of the present in vitro study, electrolytic decontamination with KI and $CHNaO_2$ was significantly more effective in reducing bacterial stained surface of rough titanium implants than air-abrasive powder, diode laser, and plasma cleaning, regardless of the accessibility of the contaminated implant location.

Clinical relevance Complete bacterial elimination (residual bacteria < 1%) was achieved only for the electrolytic cleaning approaches, irrespectively of the favorable or unfavorable access to implant surface.

Keywords Dental implants · Debridement · Electrolytes · Air abrasion · Laser therapy · Photodynamic therapy · Plasma ablation · Fluorescence microscopy · Peri-implantitis

Introduction

Peri-implantitis is an infectious disease of the tissues surrounding an osseointegrated dental implant and is currently one of the leading causes for implant failure [1, 2]. The 2017 World Workshop on Periodontal and Peri-implant Diseases and Conditions stressed the role of subgingival bacteria as the etiology of peri-implant biological complications [1, 3, 4], and the importance of triggering factors that might exacerbate plaque-induced inflammatory conditions [5, 6]. Among the multiple factors that have been described in the literature contributing to implant failure [7–9], dysbiosis of peri-implant microbiota with secondary immune complications is one of most investigated [10–13] leading to antibiofilm protocols for the success of peri-implantitis treatment.

Current therapy succeeds to lower the subgingival bacterial load to some extent without its complete elimination [14]. Undercuts between implant threads offer a protected environment for bacterial colonization and, at the same time, make impossible to completely decontaminate the implant

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surface with the current mechanical devices [15–17]. Systematic reviews concluded that open-flap debridement with mechanical instrumentation alone seemed to be insufficient to successfully treat peri-implantitis [18, 19], raising the need to for alternative treatment modalities [20–22].

One of the most investigated areas is the clinician's ability to explore and completely disinfect the contaminated implant surface [2, 23]. Multiple decontamination approaches have been proposed and tested but none of them showed adequate antibacterial efficacy to be considered as the standard of care. Air-polishing devices are largely used to remove biofilm during non-surgical and surgical therapy, as adjunct to curettes and ultrasonics. In vitro studies have proven the efficacy of air-abrasive devices to clean valley areas of the implants [24] with minimal alterations of the implant surfaces [16]. Angulation of the device tips and access into the narrow intraosseous defects are, however, limiting factors that weaken its clinical applicability.

Laser photodynamic therapy has been implemented to overcome the limitations of the physical approaches for biofilm decontamination [25]. Both in vitro [26] and in vivo studies [27] testified the antimicrobial effects of photodynamic therapy by diode-activation. However, complete bacterial elimination if often not achieved [27], and human randomized clinical trials failed to show any clinical statistically significant benefit for the adjunctive use of laser photodynamic therapy compared to standard protocols using mechanical instruments and antibiotics [28, 29].

Decontamination with plasma uses an ionized gaseous substance to achieve biofilm removal, promote cell attachment, and improve hydrophilicity of the implant surfaces [30, 31]. Whereas increased hydrophilicity would be an advantage for osteoblasts proliferation and attachment [32], antibacterial properties of plasma are suboptimal, especially for the valley between implant threads. When plasma was tested on implants specimens, 5 to 25% of the surface under the lower aspect of the threads resulted untreated even under optimal access [17].

Electrolytic cleaning is an emerging device that utilizes an electrolytic process between the implant and a counter electrode [33]. The electrolyte components are split into anions and cations. Hydrogen cations penetrate the biofilm and take an electron from the implant surface; as result, H_2 hydrogen bubbles are formed between the implant and the biofilm, that is physically detached and then destroyed in the iodine solution [34]. Pilot animal and human studies confirmed the safety and efficacy of the electrolytes, which were followed by bone gain at radiographic and clinical examination, and histological evidence of direct bone-to-implant contact [35, 36].

As the electrolytical cleaning method has shown promising reduction in bacterial colonization, it is of current interest to investigate its efficacy compared to known cleaning methods. Therefore, the aim of the present study was to test the decontamination properties of two electrolytic protocols compared to other commonly used tools as air-abrasive devices, diode laser, and plasma.

Materials and methods

Sample preparation

The present in vitro study used 60 dental implant replicas. Implants were in grade 4 titanium with screwed thread macro-design and sandblasted large-grit acid-etched surface, obtained by a turning machine (emco Austria, emcotronic TM02 and emco turn 120). Implants were 12 mm long and had a diameter of 4 mm. Two-point holes with diameter of one millimeter (mm) and a depth of 0.6 mm each were drilled in standardized positions on the implant surface and were used as measuring points for the microscopic analysis (Fig. 1). The coronal measuring point was named tested area 1 (TA1), it was located 6 mm from the apical end of the implant and it had a surface that was parallel to the implant axis (0°). The apical measuring point was named tested area 2 (TA2), located at the apical end of the implant specimen and tilted 60° to the implant axis. Both TA1 and TA2 faced the same surface of the specimens. Mean surface roughness of TA1 and TA2 was measured using a 3D laser scanning microscope (VK-X-100, KEYENCE, Osaka, Japan) and measured 1.099 ± 0.060 µm and 1.470 ± 0.150 µm, respectively.

The specimens were fabricated as one piece with the implant on one side and a cylinder on the other. The cylinder was inserted in polyether-ether-ketone (PEEK) device that was used to hold the implant during the phases of contamination. Before decontamination, a new and sterile masking

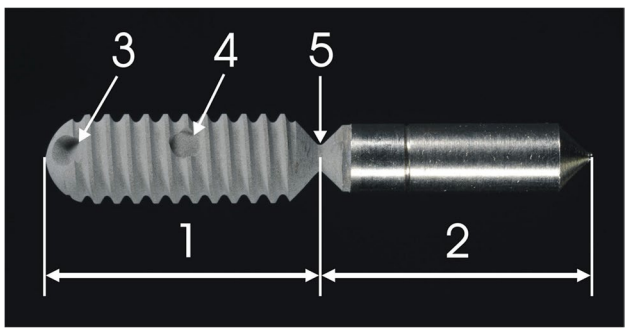


Fig. 1 Implant specimen. The implant body was 4 mm wide and 12 mm long (1). The implant was connected to a cylinder (2) long 12 mm that was used to hold the specimen during the phases of contamination and decontamination. Two test areas (TA) were used for microbial inspection and were 60° tilted (3) or parallel (4) to the implant axis. A mechanically weak neck (5) connected the implant to the cylinder

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(PEEK device) was taken to fix the specimens. Treatment phases and study workflow were reported in Fig. 2.

Incubation

Incubation of implant specimens was conducted as previously described by Ratka et al. [34] with 2 ml of unstimulated saliva that was collected from ten systemically-healthy adults by passive drooling into sterile plastic tubes. Subjects were asked to refrain from any food or drink 2 h before donating saliva and to spit directly into the saliva collection tube. Saliva samples were pooled together, to ensure that all implants were subjected to the same biofilm condition, centrifuged at 2600 g for 10 min to spin down large debris and cells, then they were incubated for 24 h at 37 °C in 250 ml Tryptic Soy Broth solution (Merck KG, Darmstadt, Germany). After incubation time, 1.5 ml of the solution was pipetted together with 0.5 ml of a freezing solution into Eppendorf tubes and frozen at − 80 °C.

The specimens, with the cylinders fixed into the PEEK devices, were placed in a prefabricated polytetrafluoroethylene (PTFE) dish, submerged with Soy Broth solution and stored into a glass container. An amount of 2 ml of the pooled human saliva solution was then added to the Tryptic Soy Broth solution under continuous shaking. To guarantee vitality of the biofilm, the broth solution was refreshed every 2 days. Implants were incubated for a total period of 14 days.

At the end of the incubation period, implant specimens were removed from the previous infected PEEK devices, mounted on a new sterile PEEK device, and prepared for the decontamination process.

Within the incubation, the 60 implants were divided into 2 groups of 30 implants, as a maximum of 36 implants could be incubated in one glass cylinder.

Decontamination modalities

Sixty implants were randomly allocated to 6 groups of ten implants each (Fig. 2). Randomization was performed with sort-random function in Microsoft Excel. Group allocation was concealed for operators involved in the debridement and microscope phases (RA, LW) and it was kept secret until the end of the experimental phases. The control group was left untreated and 5 different cleaning procedures were performed for the test groups including (1) air-abrasive powder (Air-Flow Master®, E.M.S. Electro Medical Systems S.A., Switzerland), (2) plasma cleaning (PlasmaOne®, Plasma Medical Systems GmbH, Germany), (3) diode laser (HelboLaser®, Bredent Medical GmbH & Co KG, Germany), (4) electrolytic decontamination with potassium iodide (KI), and (5) electrolytic decontamination with sodium formate ($CHNaO_2$). All treatment devices were used following the manufacturer recommendations. Under continuous turning (30 rpm) and back and forth movement along the implant (15 strokes per minute), air-flow cleaning was used at a working distance of 5 mm, with a 30° angle in relation to the implant axis and with 14-µm powder (Air-Flow® Pulver PLUS) for 2 min. The AIRFLOW Master® device was used in PERIOFLOW® mode in combination with the AIRFLOW® spray handpiece and AIRFLOW® Powder PLUS. The basis powder was erythritol with an average particle size of 14 µm. According to the manufacturer, the AIRFLOW Master® from EMS was connected to a compressed air

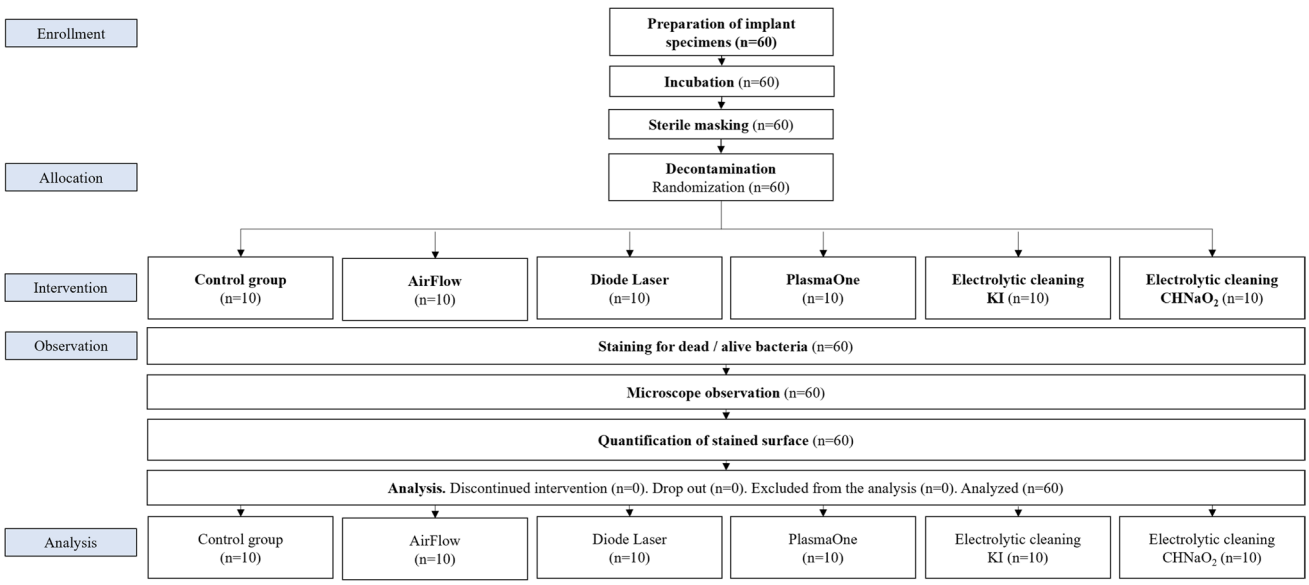


Fig. 2 Flow-chart of the study design

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supply with 6 bar. Test group 2 was debrided with PlasmaOne device. Specimens were turned and fixed in 6 positions. One of those was above the inspection areas TA1 and TA2. The treatment was carried out following manufacturer recommendation with a working distance of 2 mm at every of the 6 positions for 1 min. The PlasmaOne from plasma MEDICAL SYSTEMS® was operated in combination with the PS12 dental probes and the noble gas argon. To create a connection with the grounding of the PlasmaOne device (plasma MEDICAL SYSTEMS® GmbH), copper wires were connected to the implant test specimen below the predetermined breaking point.

Test group 3 was decontaminated with the diode laser. The diode laser (HELBO®TheraLite, brendent medical GmbH & Co. KG, Senden, Germany) emits light of a wavelength of 660 nm and has a power of 75 mV. Similar to group 2, implants were turned and fixed in 6 positions, one of them above the created point holes TA1 and TA2. Implants were moistened with a photodynamic fluid for 3 min. Then, the fiber glass tip of the diode laser was positioned at all 6 positions for 10 s.

Two different electrolytic solutions differentiated test groups 4 (potassium iodide, KI) and 5 (sodium formate, $CHNaO_2$), and both used the same physical principle of electrolytical hydrolysis method. As a counter electrode, a platinum-coated titanium wire (Ti-grade 2, Ø = 0,75 mm) was positioned in a ring (Ø = 10 mm) in a distance of 5 mm away the coronal end of the test specimens. During the cleaning process, specimens were covered with 30 ml of the electrolyte solutions (test group 4: KI 125 g, L8 + lactic acid 6 g, pure water 1000 ml, pH value 2.30, electrical conductivity 84.9 mS; test group 5: $CHNaO_2$ 200 g, L8 + lactic acid 6 g, pure water 1000 ml, pH value 5.45, electrical conductivity 117.9 mS) and a constant electric current of 600 mA was administered on the implant specimens for 3 min. Visible bubbles were developed directly on the surface of the specimens.

After the completion of each cleaning modality, procedures ended with a rinsing of the specimens with sterile water for 10 s with flow rate of 7L/min to remove all the residual chemicals and all loose contamination.

Then, the implants were carefully separated with a sterile tool from the mounting cylinder at the predetermined weakness points. Specimens were placed into Eppendorf tubes together with 2 ml of a freezing solution (phosphate buffered solution with bovine blood serum) and frozen at − 80 °C.

Microscope analysis

Within a week, specimens were unfrozen, and the Eppendorf tubes were injected with LiveDead BacLight fluorescence Kit. After staining of dead and alive bacteria (red color defined dead bacteria, green channels living bacteria), fluorescent analysis was performed at the microscope (Axioobserver, AxioCam MR3, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) and microscopic pictures were taken from TA1 and TA2. One image was created in the red and one in the green color spectrum. These two images were automatically overlaid on an image that contained red and green pixels.

Images were imported in a software for image elaboration (ImageJ1.50i; Wayne Rasband, National Institutes of Health, USA). Intensity of staining was quantified as each red and each green pixel was advised to a gray level ranging from 0 and 255.

Statistical analysis

The present in vitro study satisfies the Checklist for Reporting In-vitro Studies (CRIS). Continuous variables are reported as median [min, max]. After testing for normality with the Saphiro Wilk test, comparisons between the independent groups were performed with the Kruskal–Wallis test and for multiple comparisons we have used the Conover-Iman test, as appropriate. The level of significance was set to $\alpha = 0.05$ and all tests were two-sided. The level of significance in post hoc tests was corrected for multiple testing. Statistical analysis was performed with R 3.6.1 https://CRAN.R-project.org (R Foundation for Statistical Computing, Vienna, Austria). The R package "conover.test" version 1.1.5 was used for multiple pairwise comparisons [Alexis Dinno (2017)].

Sample size calculation

Since this is the first study to compare air-abrasive powder, diode laser, plasma cleaning, and two electrolytic test protocols using either potassium iodide (KI) or sodium formate ($CHNaO_2$) solution, there is no prior data available for a sample size calculation for ANOVA test.

As a result, a priori sample size calculation was performed using the comparison between air abrasion and diode laser [37] in G*Power (Version 3.1.9.2). A significance level (α) set to 0.05, a power (1-β) of 0.95, and an allocation ratio of 1 were used for the calculation. The effect size of biofilm quantity was 2.406, and a minimum sample size of 6 specimens per group was required. Under the assumption of a higher dispersion, 10 titanium implant units were finally used in each group for our study.

Results

A statistically significant group effect existed among decontamination protocols for both test area 1 (TA1) and test area 2 (TA2). Bacterial contamination remnants after

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each treatment modality were evaluated as residual rates of stained surface and reported in Table 1.

Statistical significance of the comparisons among treatment groups for TA1 is reported in Fig. 3. All treatment modalities showed a lower rate of stained surface compared to untreated control ($p < 0.001$ for all comparisons). The two electrolytic cleaning groups were equally effective for surface decontamination (T1 residual alive bacteria [surface %]: KI vs. $CHNaO_2$, 0.01 ± 0.02 vs. 0.008 ± 0.01, $p > 0.05$; T1 residual dead bacteria [surface %]: KI vs. $CHNaO_2$, 0.01 ± 0.02 vs. 0.005 ± 0.003, $p > 0.05$). Among the five tested protocols, both electrolytic groups were significantly more effective for removal of both dead and alive bacteria compared to AirFlow, Diode Laser, and PlasmaOne ($p < 0.001$ for all comparisons). TA1 surfaces treated with AirFlow were significantly cleaner than Diode Laser and PlasmaOne for both alive and dead bacteria ($p < 0.001$ for all comparisons). Diode Laser and PlasmaOne were the least effective modalities; Diode Laser had a significantly lower surface rate covered by alive bacteria compared to PlasmaOne ($p < 0.001$) while no difference existed on the surface rate covered by dead bacteria between the two groups.

As for TA1, also for TA2, all treatment modalities achieved a surface rate that was significantly freer from alive and dead bacteria when compared to the untreated control group ($p < 0.05$; Fig. 4). The two electrolytic cleaning groups showed the highest decontamination properties with no difference between them (T2 residual alive bacteria [surface %]: KI vs. $CHNaO_2$, 0.03 ± 0.01 vs. 0.02 ± 0.02; $p > 0.05$; T2 residual dead bacteria [surface %]: KI vs. $CHNaO_2$, 0.05 ± 0.09 vs. 0.03 ± 0.06; $p > 0.05$). Both KI and $CHNaO_2$ achieved surfaces freer from dead

Table 1 Rate of surface area positive for green-stained alive bacteria or red-stained dead bacteria. Surfaces of test areas 1 and 2 were parallel and 60° angled to the implant axes, and represented areas of facilitated and impaired access, respectively

Group	Test area 1 (surface %)			Test area 2 (surface %)		
	Alive bacteria	Dead bacteria	Total†	Alive bacteria	Dead bacteria	Total†
Control	56.16 ± 2.38	30.61 ± 3.46	86.78 ± 3.33	56.65 ± 1.23	33.29 ± 5.02	89.94 ± 4.92
AirFlow	1.73 ± 0.32	0.92 ± 0.59	2.66 ± 0.83	30.50 ± 2.17	11.62 ± 1.82	42.12 ± 3.46
Diode Laser	14.25 ± 5.47	9.20 ± 5.87	23.45 ± 7.48	24.07 ± 12.69	10.96 ± 4.46	35.04 ± 15.95
PlasmaOne	23.91 ± 2.24	9.33 ± 1.33	33.25 ± 3.01	28.43 ± 2.63	10.72 ± 1.48	39.16 ± 3.15
Electroytic cleaning-KI	0.01 ± 0.02	0.01 ± 0.02	0.03 ± 0.04	0.03 ± 0.01	0.05 ± 0.09	0.09 ± 0.10
Electrolytic cleaning-$CHNaO_2$	0.008 ± 0.01	0.005 ± 0.003	0.01 ± 0.01	0.02 ± 0.02	0.03 ± 0.06	0.06 ± 0.08

All values are expressed as mean ± standard deviation

†Total surface area counted the percentage of the surface positive for both alive and dead bacteria: sum of values Alive bacteria + Dead bacteria

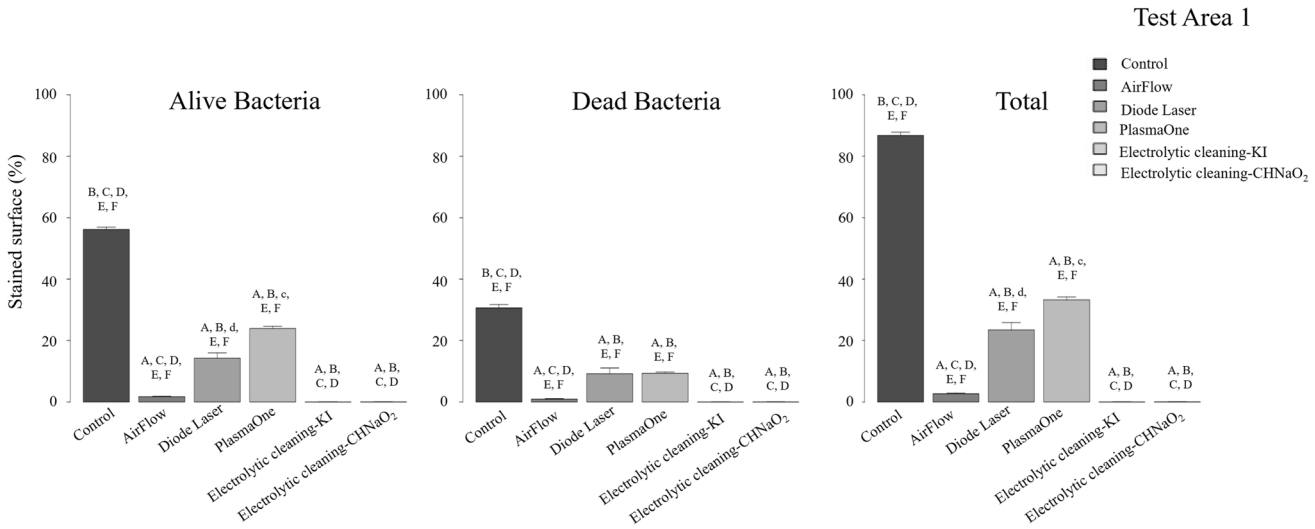


Fig. 3 Evaluation of stained surface (%) from test area 1 for alive bacteria, dead bacteria, or both. Bar graphs reported means and standard deviations. Statistical significance was reported in letters and showed a statistically significant difference compared to the control group (a), AirFlow (b), Diode Laser (**c**), PlasmaOne (d), electrolytic cleaning-KI (e), or electrolytic cleaning-CHNaO2 (f). Capital letters were used for $p < 0.001$; small letters were used if $p < 0.05$

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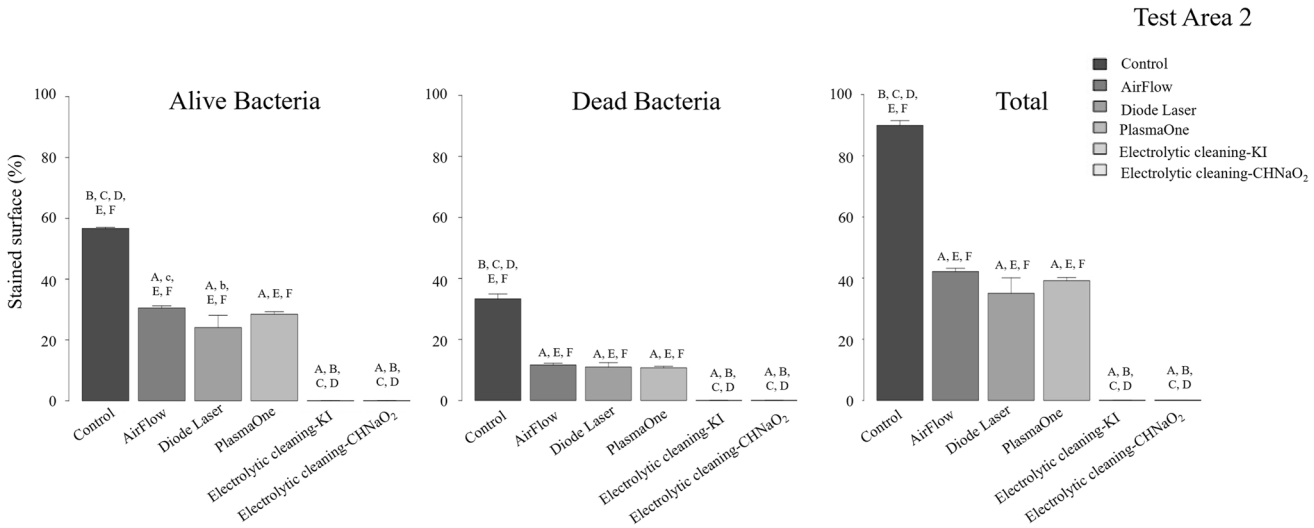


Fig. 4 Evaluation of stained surface (%) from test area 2 for alive bacteria, dead bacteria, or both. Bar graphs reported means and standard deviations. Statistical significance was reported in letters and showed a statistically significant difference compared to the control group (a), AirFlow (b), Diode Laser (c), PlasmaOne (d), electrolytic cleaning-KI (e), or electrolytic cleaning-CHNaO2 (f). Capital letters were used for $p < 0.001$; small letters were used if $p < 0.05$

and alive bacteria than the remaining treatment modalities ($p < 0.001$ for all comparisons). AirFlow, Diode Laser, and PlasmaOne were equally ineffective when tested for TA2, with Diode Laser performing better than AirFlow only for alive bacteria ($p < 0.05$).

Total stained surface was calculated as the sum of residual staining for alive plus dead bacteria, and it was used to test the efficacy of the treatment modality to physically detach the biofilm from the implant surface, regardless from the vitality of the microorganisms. Only the electrolytic cleaning groups reduced the total bacteria-stained surface to a level lower than 0.1% without statistically significant differences between TA1 and TA2 (TA1 vs. TA2; KI: $0.03 \pm 0.04\%$ vs. $0.09 \pm 0.10\%$, $p > 0.05$; $CHNaO_2$: $0.01 \pm 0.01\%$ vs. $0.06 \pm 0.08\%$, $p > 0.05$). The electrolytic cleaning groups were significantly more efficacious than other decontamination devices for reduction of total stained surface ($p < 0.001$ for all comparisons). AirFlow was significantly less efficacious in TA2 compared to TA1 (TA1 vs. TA2; $2.66 \pm 0.83\%$ vs. $42.12 \pm 3.46\%$; $p < 0.001$) and left a total stained surface area that was consistently greater than the electrolytic cleaning groups ($p < 0.001$ for all comparisons). AirFlow decontaminated significantly more compared to Diode Laser and PlasmaOne only for TA1 ($p < 0.001$ for all comparisons) but not for TA2. Diode Laser and PlasmaOne were equally ineffective in TA1 and TA2 as they left the residual colored surface always greater than one fifth of the total surface area (TA1 vs. TA2, Diode Laser: $23.45 \pm 7.48\%$ vs. $35.04 \pm 15.95\%$, PlasmaOne: $33.25 \pm 3.01\%$ vs. $39.16 \pm 3.15\%$). A statistical significance also existed between TA1 and TA2 for the PlasmaOne approach, with better decontamination for TA1 compared to TA2 ($p < 0.001$).

Discussion

Pathogenic bacteria have been regarded as the primary etiology of peri-implantitis lesions [1] and their elimination represents the goal of successful therapeutic and preventive treatment modalities [11, 38]. Currently used debridement protocols showed to be inadequate for complete surface decontamination and are often followed by suboptimal clinical outcomes [18, 19]. Therefore, it is of high interest for the field to develop new tools to achieve complete elimination of the biofilm attached to the implant surface.

In the present investigation, the electrolytic cleaning was the most efficacious treatment to completely decontaminate moderately rough implant surfaces after a bacterial incubation period of 2 weeks. The electrolytic treatment was the only approach that reduced the bacterial stained surface as low as less than 0.1%. Also, it was not influenced by the surface angulation since no difference was noted between stained surface of TA1 and TA2. Among the advantages of the electrolytic protocol, there is the possibility to accessing the infected surface by fluid without the physical insertion of a cleaning device. Hydrogen cations and H_2 bubbles are then used to physically lift and destroy the microorganisms from the implant surfaces [34]. As result, no chemicals with medicinal or bactericidal effects are required to successfully remove the biofilm if electrolytic treatment is adopted. Furthermore, the two used electrolytes showed similar

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decontamination efficacy. This finding supports the concept that, in the electrolytic cleaning, the decontamination is achieved primarily through the mechanical detachment due to the formation of hydrogen bubbles between the implant and the biofilm. These findings are confirmed in recent animal and human studies that documented the safety of the electrolytic decontamination as well as favorable clinical outcomes with histological evidence of re-osseointegration [35, 36].

Air-abrasive protocol showed excellent decontamination properties only if used in areas of favorable access (TA1). On angled surfaces (TA2), however, remnants of vital plaque were noted for about 30% of the treated area, raising arguments on the ability of the air-abrasive powder to decontaminate thread undercuts in real clinical setting. Even on the open horizontal measuring points (TA1), the air-abrasive protocol left about 2% of vital bacteria, that was statistically significantly more compared to the 0.01% of the two electrolytic groups ($p < 0.001$). The in vitro study by Keim et al. [39] supports that the efficacy of air-abrasive tools is highly influenced by the decontamination angle. Implants stained with ink and treated with air abrasion showed a residual staining of 8.03% when the air-abrasive device was used with an angle of 30°, and less than 1% when the angle was 60° or 90°. Of notice is that Keim et al. [39] reported the angle between the jetted powder and the implant axes that, because of the existence of threads and valley, does not necessarily correspond to the tangent angle between the jetted powder and the implant surface. Also, the differences between Keim's and our results can be explained by the different methodology of contamination (colored ink vs. human biofilm), and implant surface treatment (machined collar and anodized surface vs. full SLA surface). Clinical application of air-abrasive devices is limited by the need to physically insert the device tip inside an infrabony defect and decontaminate valleys between threads. While decontamination of coronal surfaces using air abrasion showed favorable results [40, 41], its efficacy to treat the apical third of implant surfaces seems limited [42].

Plasma and diode photodynamic laser therapy were the least effective to decontaminate implant surface. Under ideal circumstances, roughly 15 to 25% of vital bacteria was always detectable. Current literature on plasma and laser therapy supports their advantages for osteoblasts adhesion [30] or peri-implantitis degranulation [43]. However, the pure bactericidal property of plasma and diode laser seemed to be arguable [17, 44].

The strengths of this study include the use of implant-like specimens with screwed thread macro-designs and sandblasted and acid-etched surface, while similar studies used titanium disks [45]. In addition, measuring points were standardized and simulated areas of favorable (TA1) and impeded (TA2) access, where comparable studies used straight surfaces only [45, 46]. Implants were incubated 2 weeks for bacterial contamination to allow adequate maturation of a complex pathologic biofilm. Compared to similar studies, the present investigation tested the decontamination protocols on a vital biofilm instead of on plaque-simulated colored spray, resin, or ink [16, 39].

A limitation of the study is the use of a saliva model that does not mimic the subgingival microbiota of peri-implantitis environment, as the decontamination systems selected for the study were aiming for bacterial elimination irrespectively from the bacterial species. The impossibility to realize a subgingival biofilm mitigates the strength of translation of the study in clinical practice. The present study also retains the same limitations of other in vitro studies, including the fortunate accessibility created by openness of the test specimens and the opportunity to clean the infected surfaces from all angles. This study assessed the ability of tested devices to reach and clean the apical third of contaminated implants; however, from a clinical perspective, bone loss extending to the apical third of the implant often represents an indication for explantation.

Further research is needed to evaluate the favorable outcomes reported in this study by electrolytic cleaning methods in more realistic clinical scenarios, in vitro and in vivo. Future research will also investigate the biological compatibility of the decontaminated surfaces for repopulation by osteoblasts, gingival fibroblasts, and epithelial cells.

Conclusions

Within the limitations of the present study and the used systems, all treatment modalities were able to decontaminate implant surfaces with the electrolytic protocols being the most efficacious, plasma, and diode photodynamic laser therapy the worst, and air-abrasive powder in between. Unfavorable access appeared to increase bacterial remnants, with the air abrasion being affected the most. Electrolytic cleaning is a novel promising approach for complete bacterial elimination on rough implant surfaces.

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Author contribution All authors significantly contributed to the realization of the manuscript and approved the last version before submission. HZ: research design, experiments, data acquisition, finalizing the writing, approval for submission. PW: research design, experiments, finalizing the writing, approval for submission. RG: analysis and interpretation of data, drafting and finalizing the writing, approval for submission. LS: analysis and interpretation of data, drafting and finalizing the writing, approval for submission. DH: research design,

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experiments, finalizing the writing, approval for submission. HLW: analysis and interpretation of data, finalizing the writing, approval for submission. MS: research design, experiments, data acquisition, finalizing the writing, approval for submission. CR: research design, experiments, data acquisition, finalizing the writing, approval for submission.

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Data availability The data sets used and analyzed during the current study are available as electronic supplementary material.

Declarations

Ethics approval Ethics approval was not required for this in vitro study.

Conflict of interest HZ and MS hold patent for the described technology and received funding by Zyfoma GmbH (Weiterstadt, Germany) for the realization of the study. All other authors reported no conflict of interests.

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