European Annals of Dental Sciences
Official Publication of Ankara University, Faculty of Dentistry

EADS, 2024, 51 (3), 89–95

e-ISSN : 2757-6744
Article Received/Accepted : February, 27 2024 / August, 31 2024
doi : 10.52037/eads.2024.0015

ORIGINAL RESEARCH ARTICLE

Comparison of the Effects of Different Decontamination Methods on Staphylococcus Aureus Biofilm

Ece Açıkgöz Alparslan [1], *, Ufuk Bağcı [2] and Ayşegül Kurt [3]

[1]Trakya University, Faculty of Dentistry, Department of Periodontology, Edirne, Türkiye and [2]Trakya University, Faculty of Engineering, Department of Food Engineering, Edirne, Türkiye and [3]Trakya University, Faculty of Dentistry, Department of Prosthodontics, Edirne, Türkiye

*Corresponding Author; eceacikgoz@trakya.edu.tr

Abstract

Purpose: The current in-vitro study aims to compare the effectiveness of various mechanical decontamination modalities in the elimination of Staphylococcus aureus biofilm from titanium surfaces using qualitative and quantitative techniques.
Materials and Methods: A total of 78 titanium discs were inoculated with Staphylococcus aureus and randomly allocated into control and three experimental groups consisting of plastic curettes (PC), ultrasonic-driven plastic tips (UPT), and ultrasonic-driven stainless-steel tips (UST). Following decontamination procedures, colony-forming units and viable biomass were analyzed to identify the biofilm removal efficacy of the treatments and the viability of bacteria remaining on the surface. Biofilm structure was assessed by confocal laser scanning microscopy and scanning electron microscopy. Analysis of variance and post hoc Tukey tests were applied for comparisons.
Results: Reductions in both colony counts and variable biomass following the decontamination procedure were significant in all treatment groups (p=0.000). Although the highest reduction in colony count was determined in the UST and the lowest in the PC group, the difference was not statistically significant between treatment groups (p = 0.246). Nonetheless, the reduction in viable biomass in the UST group was greater than in the UPT and PC groups (p=0.005, p=0.000, respectively).
Conclusions: Ultrasonic-driven instruments are more effective than plastic curettes in removing the biofilm that colonizes the titanium surfaces in the initial stages. Stainless steel tips provide better elimination of microbial biofilm compared to plastic instruments, but they alter the surface topography of roughed titanium surfaces more than plastic curettes.

Keywords: Biofilm; Colony forming units; Confocal Laser Scanning Microscopy; Decontamination; Peri-Implantitis

Introduction

Peri-implantitis is a site-specific lesion characterized by loss of surrounding bone and clinical inflammation of the peri-implant mucosa, potentially leading to implant loss if appropriate treatment is not provided. [1,2] Early eradication of the microbial biofilm formed by pathogens such as Staphylococcus aureus (S. aureus), which colonize the peri-implant tissues, is crucial for implant survival. [3,4]

Although it has been suggested that the bacterial biofilm on implant and dental surfaces is similar, recent data show that the microbiota in peri-implantitis is a polymicrobial anaerobic infection and has a more complex structure than that in periodontitis. [5] Many studies have shown that microorganisms such as Staphylococcus aureus, Enterobacteriaceae, Candida albicans, and Pseudomonas aeruginosa, which are not commonly found in the oral flora, can be present in the peri-implant flora. These microorganisms are rarely associated with periodontal disease but can successfully adhere to titanium surfaces. [5,6] The strong affinity of S. aureus for titanium surfaces and its ability to adhere to extracellular matrix components accumulated on biomaterial surfaces play a critical role in biofilm formation, leading to implant-associated infections. [7] S. aureus, one of the microorganisms involved in peri-implant diseases, can colonize the implant surfaces in the early stages after implant placement. [3] Additionally, it is known that individuals with failed titanium dental implants have low titers of antibodies against S. aureus. [6,7] Therefore, the complete removal of early colonizers like S. aureus from dental implant surfaces could determine long-term implant success.

Various mechanical techniques for biofilm removal have been proposed in the literature. [8] Ultrasonic instruments and curettes made of various materials are frequently utilized for this purpose. [9] Among the mechanical methods used in daily practice, ultrasonic instruments are probably the most commonly used for biofilm removal on both implant and dental surfaces due to the ease of use

How to cite: Acikgoz Alparslan E, Bagci U, Kurt A. Comparison of the Effects of Different Decontamination Methods on Staphylococcus Aureus Biofilm. EADS. 2024;51(3): 89-95

provided by the micro-movements of the ultrasonic tips.[10,11] However, the results from studies on the effectiveness of biofilm removal using ultrasonic tips have been controversial. While some studies reported that ultrasonic tips outperformed other mechanical techniques, regardless of the tip material (peek or steel)[12,13], others have shown contradictory results.[14,15] On the other hand, curettes made of plastic material have been introduced, considering the potential hazard that ultrasonic tips can cause to the implant surface.[16,17] However, questions remain regarding the efficacy of plastic curettes in the decontamination of implant surfaces when compared to other mechanical methods.[18–21]

Different techniques have been used to measure the remaining biofilm on titanium implant surfaces after biofilm removal methods in the literature. The use of other methods based on the principle of staining live-dead cells and imaging them with microscopes, along with traditional methods such as enumeration of viable cells by colony forming units (CFU), where only quantitative information about living cells is obtained, offers new perspectives in the evaluating results. This is because qualitative information, as well as quantitative information, is obtained from the stained cells.[22]

Clinicians face different challenges in selecting the best treatment for patients with peri-implant diseases, as there is no consensus on which mechanical technique is most effective in the elimination of peri-implantitis-causing biofilm on implant surfaces.[8] In this context, the present study aims to compare the effectiveness of various mechanical decontamination modalities in the removal of Staphylococcus aureus biofilm from titanium implant surfaces, using qualitative and quantitative methods. The null hypothesis is that the evaluated mechanical techniques will yield comparable outcomes in terms of the biofilm elimination potential.

Material and Methods

The study was conducted using 78 titanium discs (Ø 6 mm and a thickness of 4 mm) produced from dental implant material whose surface was roughened with biphasic calcium phosphate (Bioinfinity, Istanbul, Türkiye). Gamma irradiation was used by the manufacturer to sterilize the titanium disc specimens.

Biofilm formation

All sterile discs were covered with Staphylococcus aureus (S. aureus) biofilm.[23] Reference strain S. aureus ATCC 29213 was preferred to establish a bacterial biofilm layer on disc surfaces. The S. aureus ATCC 29213 was cultured at 37 °C for 24 h using brain heart infusion broth (BHI, Merck, Germany). Cells were then diluted in BHI until an optical density at 600 nm of 0.1 was reached using a spectrophotometer (UV-1800 Shimadzu, Japan). The bottom and side surfaces of the titanium discs were carefully covered with parafilm to ensure that the biofilm formation occurred only on the upper surface of the discs. Then, the parafilm-covered discs were placed into the wells of a 24-well flat bottom plate, and 1 mL of the bacterial culture was transferred to each well. The plates were incubated statically (37 °C, 48h) to establish an intact biofilm layer on the titanium discs.

At the 24th hour of incubation, the culture medium in the wells was carefully removed with a pipette, and 1 mL of sterile BHI broth was added to the wells. The discs were taken out of the wells following the incubation and were gently cleaned by washing them three times with 2 mL phosphate-buffered saline (pH 7.4) to eradicate any planktonic or loosely adherent bacteria that were not embedded in the biofilm.[24,25]

Experimental design

The total number of bacteria-coated titanium discs used in all stages of the study was 78 (n=42 for CFU analysis, n=28 for confocal microscopy and n=8 for scanning electron microscopy). The study comprised three experimental groups, each subjected to different disinfection methods, as follows: 1) Ultrasonic-driven steel tip: An ultrasonic device with a stainless-steel tip (Air-Flow Master Piezon with PS instrument, EMS, Nyon, Switzerland) was used at the 80% power and maximum water cooling.[26] 2) Ultrasonic-driven plastic tip: The scaling was performed using a thermoplastic scaler tip (Air-Flow Master Piezon® with PI instrument, EMS, Nyon, Switzerland) made of polyether ether ketone (PEEK) material with the same settings (power 80%, water 100%) recommended by the manufacturer. 3) Plastic curette: The surface of the previously contaminated discs was decontaminated using a hand-instrument made from high-grade resin (ImplacareTM II; Hu-Friedy®; Chicago, IL, USA).

Bacteria-coated titanium discs receiving no treatment served as controls. All titanium discs belonging to the experimental groups were decontaminated by the same experienced operator. During decontamination procedures, the angulation and working distance of the instruments were adjusted by the operator to ensure optimal access to the disc surface. The working tip of the instruments was contacted the disc surface without pressure. Instrumentation time was limited to 2 min for each decontamination procedure and controlled using a stopwatch. Following instrumentation, remnants were cleaned from the treated surfaces by gentle rinsing with distilled water for 20 s.[13] During the procedure, sterile instruments and materials were used to prevent contamination of the titanium surfaces with microorganisms other than S. aureus.

Analysis by Colony Forming Unit (CFU) counting

A total of 42 bacteria-coated discs were used for CFU analyses and 14 bacteria-coated titanium discs were allocated to each experimental group with 7 designated for treatment and 7 for control.[27] Due to the complexity of the applied procedure (CFU counting process), separate control groups were created for each experimental group, and each was compared with its own control.

The quantity of S. aureus on treated surfaces was calculated in CFU per titanium disc, allowing for a quantitative evaluation of the remaining biofilm. Enumeration of S. aureus ATCC 29213 in the control (untreated) and treated samples was performed using the surface spread technique. For this purpose, titanium discs were transported to Falcon tubes containing 10 mL of 0.5% (w/v) Tween20 PBS. Subsequently, the tubes were vortexed for 1 minute and sonicated (35 kHz, Sonorex, Germany) for 5 minutes at 25°C to disrupt the biofilm. After sonication, the tubes were vortexed for another minute, and decimal serial dilutions were prepared using sterile saline solution (0.85%, w/v). Then, 100 µl of each dilution was spread onto Tryptic Soy Agar plates, and incubated (48 h at 37°C).[28]

The reduction in colony count (CFU/surface) was calculated using the following equation: R= (C−T)

Where C is the number of colonies in control samples (no treatment), T is the number of colonies after the treatment and R is the log reduction in colony count (CFU/surface).

Analysis by Confocal Laser Scanning Microscopy (CLSM)

A total of 28 bacteria-coated titanium discs were immunostained for confocal microscopy, and randomly divided into a control group and three experimental groups that received different disinfection modalities. Titanium discs were placed in wells of flat bottom plates and stained with the LIVE/DEAD BacLight Bacterial Viability and Counting Kit (Invitrogen, Merelbeke, Belgium), subsequently left under light protection for 15 min. Confocal laser scanning microscopy (Leica Lasertechnik, Heidelberg, Germany) was used to examine three randomly selected fields on each specimen. Excitation wavelengths were set as 488 and 532 nm, and 10/1.0 magnification optical lenses were preferred for observing the specimens. The

Table 1. Mean reduction values in colony counts and viable biomass according to the decontamination procedures

Group	Colony Count in Control Groups (CFU/Surface)	Log Reduction in Colony Count (CFU/Surface)	p	Viable Biomass in Control Group	Reduction in Viable Biomass
Ultrasonic-driven steel tip (UST)	5,33±0,14	1,39±0,42	**0.000a***	18,01±4,62	15,82±0,9A
Ultrasonic-driven plastic tip (UPT)	6,14±0,24	1,14±0,54	**0.000a***		7,57±5,85B
Plastic curette (PC)	6,36±0,18	1,02±0,12	**0.000a***		1,35±4,35C
		0.246b			**0.000b***

a: Independent samples test, b: Analysis of variance (ANOVA), * p<0,05. A shows the statistically significant differences between the reduction in viable biomass in the ultrasonic-driven steel tip group and the reductions observed in the ultrasonic-driven plastic tip and plastic curette groups, Tukey's test (p=0.005 for UST- UPT groups, p=0.000 for UST-PC groups); B shows the statistically significant differences between the reduction in viable biomass in the ultrasonic-driven plastic tip group and the reductions observed in the ultrasonic-driven steel tip and plastic curette groups, Tukey's test (p=0.005 for UPT- UST groups, p=0.035 for UPT-PC groups); C shows the statistically significant differences between the reduction in viable biomass in the plastic curette group and the reductions observed in the ultrasonic-driven plastic tip and ultrasonic-driven steel tip groups, Tukey's test (p=0.035 for PC-UPT groups, p=0.000 for PC-UST groups).

properties of the total and viable biomass (m3/m2) were measured using the COMSTAT software.

Analysis by Scanning Electron Microscopy (SEM)

Two discs from each group were randomly allocated following the instrumentation for SEM evaluation. Biofilm on the titanium surface was fixed for one hour with glutaraldehyde (2.5%) and dehydrated with multiple ethanol washes (10%, 25%, 50%, 75%, and 90% for 20 m, and 100% for one hour). Following the dehydration of the biofilm was accomplished, titanium disc specimens were kept in the incubator at 37 °C overnight. Gold coating was applied to the specimens, which were carefully examined using an SEM (Apreo S, ThermoFisher Scientific, Norway) at 10 kV and magnifications of 1000, 2500, 5000 and 10.000x. Representative micrographs of S. aureus biofilm remaining attached to titanium surfaces were taken, and descriptive analysis of these images was conducted.

Statistical analysis

In the power analysis conducted before the study (80% power and probability of error α = 0.05), the sample size for each study group was determined as 12 titanium discs. IBM SPSS Statistics software (Version 23.0, SPSS Inc., Chicago, IL, USA) was used to analyze data. Shapiro-Wilk test was performed to determine the distribution of the data. Normally distributed data was submitted for analysis of variance and post hoc Tukey tests to assess dissimilarities among the study groups. The Pearson correlation coefficient was used analyze the relationship between colony counts and viable biomass values. Descriptive statistics were given as mean ± standard deviation. The level of .05 was accepted as the limit of statistical significance.

Results

Mean reductions in viable log10 counts and viable biomass according to the treatment procedures were presented in Table 1.

Reductions in S. aureus colony counts following the decontamination procedure were significant in all treatment groups (p=0.000). The highest log reduction in colony count was determined in the ultrasonic-driven steel tip (1,39 ±0,42 log CFU/surface) and the lowest was in the plastic curette group (1,02 ±0,12 log CFU/surface, Table 1). However, no statistically significant difference was noted among the decontamination modalities (p=0.246).

Viable biomass values confirmed by COMSTAT quantification showed a significant decrease on all treated discs (p=0.000). Consistent with the log reduction in the colony counts, the highest decrease in the viable biomass was detected in the ultrasonic-driven steel tip group (15,82 ± 0,9), while the lowest decrease was in the plastic curette group (1,35 ± 4,35), (Table 1). According to the variance analysis, there was a significant difference in the reduction of viable biomass between the groups (p=0.000). The reduction in viable biomass in the ultrasonic-driven steel tip group was higher than in the ultrasonic-driven plastic tip and plastic curette groups (p=0.005, p=0.000, respectively).

Figure 1 revealed the presence of the remaining S. aureus biofilm attached to the titanium surface. CLSM images of control discs confirmed the predominance of live bacteria on untreated disc surfaces. The quantity of live bacteria was lowest in the ultrasonic-driven steel tip group and highest in the plastic curette group. In all treatment groups, the quantity of live bacteria dominated the dead bacteria (Figure 1).

In SEM micrographs, S. aureus colonies were more pronounced in untreated control discs and plastic curette-treated discs. However, although ultrasonic-driven procedures were more efficient in microbial biofilm removal, they altered the surface topography of the discs. It has been noted that the initial roughened surface of the discs is damaged in all treatment groups. Moreover, plastic remnants were detected on the surfaces treated with plastic instruments (Figure 2).

Discussion

Current treatment modalities are focused on surface decontamination methods to eliminate the biofilm attached to the titanium surface since the main causative factor of peri-implantitis is a pathological biofilm.[26] Hand instruments and ultrasonic devices are widely used due to their ease of daily practice and lower costs compared to other mechanical methods.[29] This in vitro study provides a comparative evaluation of the effect of three frequently preferred decontamination methods in daily practice on the removal of S. aureus biofilm from previously contaminated titanium disc surfaces.

In the present study, significant reductions were observed in both colony count and viable biomass of S. aureus in all treatment groups compared to the control. In agreement with our findings, Kawashima et al.[10] reported that both the steel tip and the plastic-coated tip significantly removed microbial biofilm from the implant surface.

Among the three decontamination modalities investigated, ultrasonic-driven steel tips were found to be more effective than ultrasonic-driven PEEK tips and plastic curettes. Our viable biomass results revealed that this observed difference was statistically significant. These findings are consistent with studies indicating plastic curettes as less efficient in removing the biofilm layer compared to other mechanical decontamination modalities.[20,30] Besides these studies, Schmage et al.[31] demonstrated that two types of ultrasonic scalers with steel and a plastic-coated tip had better biofilm removal scores than plastic curettes. Unlike our results with viable biomass,

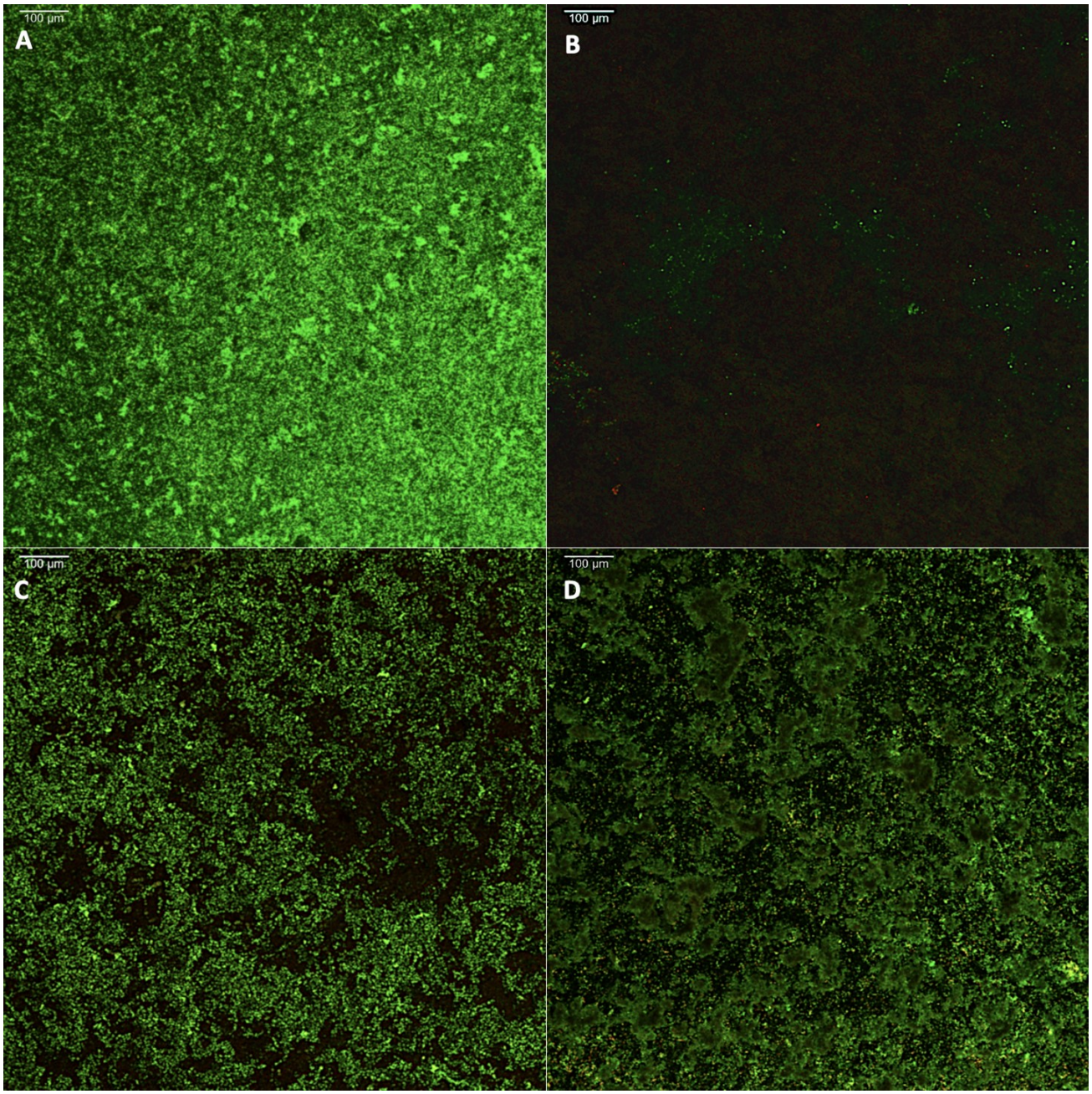

Figure 1. Representative CSLM images of S. aureus biofilm on the surfaces of control and treatment discs: (A) Control, (B) Ultrasonic-driven steel tip, (C) Ultrasonic-driven plastic tip, (D) Plastic curette (Viable bacteria = green; dead bacteria = red).

Luengo et al.[14] did not observe any difference in decontamination effectiveness between ultrasonic-driven steel tips and PEEK tips, both macroscopically and microscopically. They attributed this to the inability of the ultrasonic tips to reach the valley parts of the implant threads and perform effective decontamination. The discrepancy in our study results may be ascribed to using flat-surfaced titanium discs rather than the original threaded implant surface.

On the other hand, no significant difference was detected among the treatment groups according to our colony count results. Similarly, in a study conducted by Renvert et al.[32], the effectiveness of ultrasonic systems compared to manual curettes was investigated and no significant difference was reported. Another study comparing ultrasonic instruments and plastic curettes also demonstrated no significant difference in results obtained with either method.[33] Furthermore, Kawashima et al.[10] stated no significant difference in biofilm removal from titanium implant surfaces between plastic-coated tipped and steel-tipped ultrasonic devices. All these studies, including ours, overlook the nature of the oral cavity where the implant is placed. Considering the pH balance, temperature, and humidity of the oral environment, the contribution of saliva to bacterial biofilm formation, and the challenges of removing biofilm from implant surfaces, statistical differences between these decontamination techniques can be expected in further clinical studies.

SEM images of the present study reveal that the ultrasonic-driven steel tip nearly eliminates the S. aureus biofilm from the surface but alters the surface structure severely. Similarly, in a study that evaluated SEM images, Schmidt et al.[34] presented that treatment groups containing stainless steel instruments caused more detrimental changes on the implant surface than other treatment groups. Additionally, in their study, which is very similar to ours and includes SEM images, Beak et al.[35] observed that conventional steel tips caused more damage to titanium surfaces compared to plastic-coated tips. When ultrasonic devices are used, the oscillation of the steel ultrasonic tip effectively removes the biofilm on the

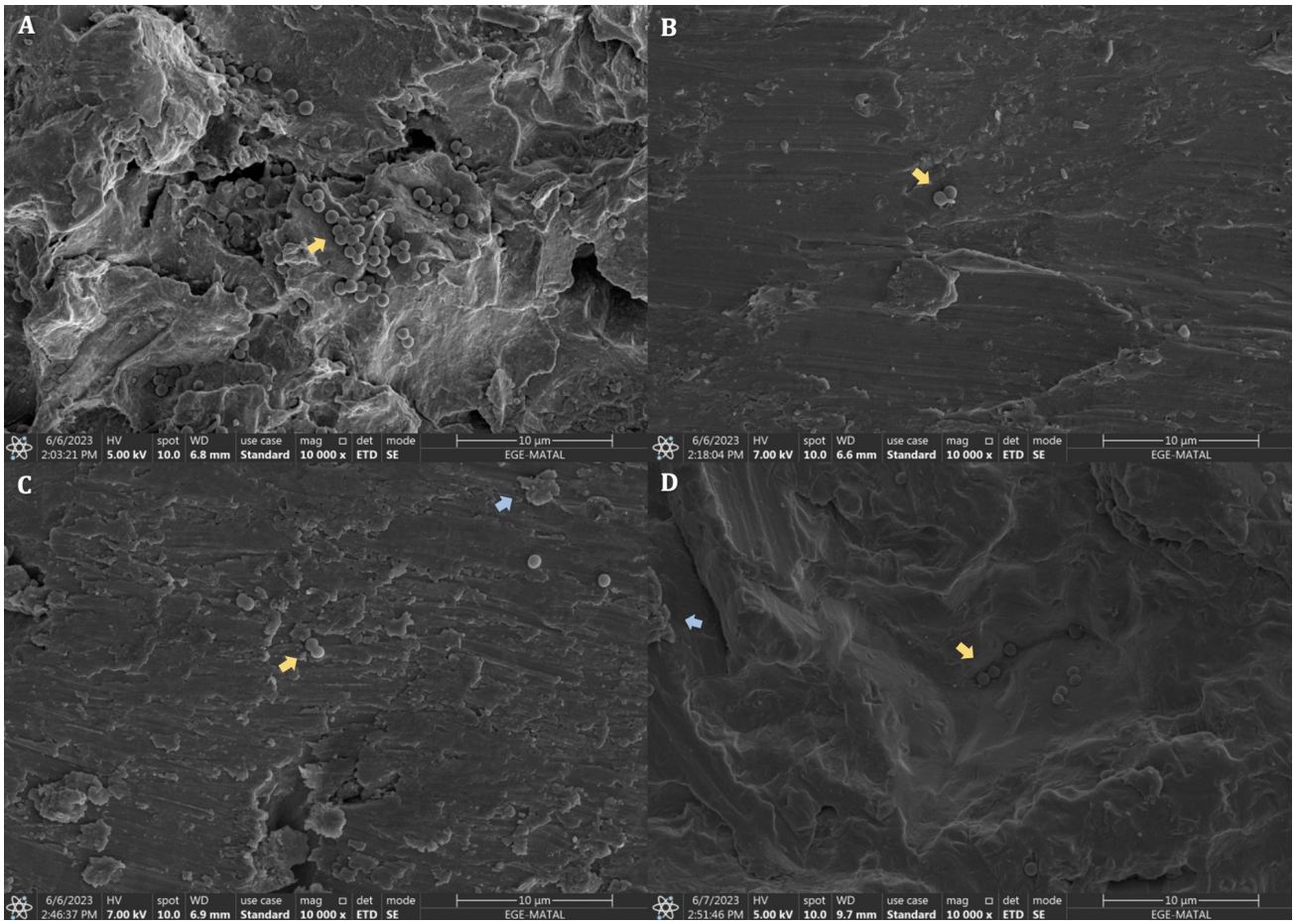

Figure 2. Representative SEM images of the S. aureus biofilm on the disc surfaces following decontamination procedures at a magnification of × 10000: (A) Control, (B) Ultrasonic-driven steel tip, (C) Ultrasonic-driven plastic tip, (D) Plastic curette. S. aureus biofilm (yellow arrow), and plastic remnants (blue arrow) remaining on the treated surfaces are indicated by arrows.

implant surface, yet this effect may come at the expense of damaging surface integrity.[15,26] Alternative tips for ultrasonic-driven devices, such as plastic coating tips, have been demonstrated to leave remnants of coating material on the implant surface.[36,37] Consistently to the mentioned studies, we observed that ultrasonic-driven plastic tips altered the surface integrity and left plastic remnants on the surface.

A plastic curette could be preferred when the main goal of treatment is to maintain surface integrity; but its capability to effectively eliminate microbial biofilm from implant surfaces has been widely questioned.[37] SEM images from our study indicate that the detrimental effect of plastic curettes on surface integrity was insignificant compared to ultrasonic tips. Consistent with the other findings of this study, we observed that the plastic curette was insufficient in removing biofilm compared to other decontamination modalities and left plastic remnants on the surface of the titanium discs. Similarly, Hakki et al.[38] detected plastic residues on the plastic curette-treated implant surfaces. In another similar study, plastic remnants were detected on all titanium surfaces following treatments performed with different plastic instruments. Still, the amount of remnants was highest on plastic curettes.[39]

The present study has several limitations. One limitation is that titanium discs were tested instead of screw-shaped implants to ensure standardization in assessing bacterial elimination. Although roughened titanium discs have the same microstructure as the original implant surfaces, decontamination of the implants is much more complicated cause of the presence of valleys between the threads. This screw-shaped design of titanium implants may hinder instruments from accessing the diseased surface and limit decontamination procedures. Another limitation is that an in vitro S. aureus biofilm is preferred over a microbial biofilm with a complex structure that may be more resistant to instrumentation. The findings of this study revealed the incapability of all tested treatment procedures to total removal of S. aureus biofilm, so it can also be estimated that it would be ineffective in eliminating more pathological biofilm.

Within the limitations, mechanical decontamination procedures evaluated in this study presented some beneficial effects in the removal of S. aureus biofilm from titanium surfaces. However, none of these methods were sufficient to eliminate the biofilm. These findings indicate that instrumentation of S. aureus-infected titanium surfaces with mechanical procedures alone may not be sufficient to eradicate the intact biofilm. This is consistent with studies reporting that the combined use of mechanical and chemical methods or other newly developed instruments like lasers can increase the disinfection effect.[18,33] Additionally, alterations in the surface topography and surface chemistry of the implant have a significant efficacy on bacterial biofilm formation.[40] Therefore, further investigations on surface properties and bacterial elimination methods are required before a definitive treatment recommendation can be provided.

Conclusion

All investigated procedures resulted in reductions in the quantity and viable biomass of S. aureus biofilm on titanium surfaces, but none of them achieved complete elimination of the biofilm. To establish a gold standard method capable of completely eradicating

S. aureus biofilm, attention should be directed toward combination procedures that integrate various techniques designed to minimize damage to the titanium surface while inducing chemical disruption of the biofilm.

Acknowledgements

Authors would like to thank Bioinfinity Implants (Istanbul, Turkey) for supporting this research by providing titanium disc specimens.

Financial Support

This study was funded by the Trakya University Scientific Research Projects, (Project Number: 2021/89).

Author Contributions

Conception and Design : All Authors
Data Collection : E.A.A. , U.B.
Data Analysis : A.K.
Data interpretation and Literature review : All Authors
Draft of the manuscript : E.A.A.
Final Review and Editing : All Authors

Conflict of Interest

The author declare no conflict of interest.

Authors' ORCID(s)

E.A.A. 0000-0003-4560-6156
U.B. 0000-0002-1511-2465
A.K. 0000-0003-2775-3179

References

1. Alcoforado GA, Rams TE, Feik D, Slots J. Microbial aspects of failing osseointegrated dental implants in humans. J Parodontol. 1991;10(1):11–18.
2. Berglundh T, Jepsen S, Stadlinger B, Terheyden H. Peri-implantitis and its prevention. Clin Oral Implants Res. 2019;30(2):150–155. doi:10.1111/clr.13401.
3. Fürst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007;18(4):501–508. doi:10.1111/j.1600-0501.2007.01381.x.
4. Mombelli A, Lang NP. Microbial aspects of implant dentistry. Periodontol 2000. 1994;4:74–80. doi:10.1111/j.1600-0757.1994.tb00007.x.
5. Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. Clin Implant Dent Relat Res. 2014;16(6):783–793. doi:10.1111/cid.12052.
6. Albertini M, López-Cerero L, O'Sullivan MG, Chereguini CF, Ballesta S, Ríos V, et al. Assessment of periodontal and opportunistic flora in patients with peri-implantitis. Clin Oral Implants Res. 2015;26(8):937–941. doi:10.1111/clr.12387.
7. Salvi GE, Furst MM, Lang NP, Persson GR. One-year bacterial colonization patterns of Staphylococcus aureus and other bacteria at implants and adjacent teeth. Clin Oral Implants Res. 2008;19(3):242–248. doi:10.1111/j.1600-0501.2007.01470.x.
8. Cosgarea R, Roccuzzo A, Jepsen K, Sculean A, Jepsen S, Salvi GE. Efficacy of mechanical/physical approaches for implant surface decontamination in non-surgical submarginal instrumentation of peri-implantitis. A systematic review. J Clin Periodontol. 2023;50 Suppl 26:188–211. doi:10.1111/jcpe.13762.
9. Meyle J. Mechanical, chemical and laser treatments of the implant surface in the presence of marginal bone loss around implants. Eur J Oral Implantol. 2012;5 Suppl:71–81.
10. Kawashima H, Sato S, Kishida M, Ito K. A comparison of root surface instrumentation using two piezoelectric ultrasonic scalers and a hand scaler in vivo. J Periodontal Res. 2007;42(1):90–95. doi:10.1111/j.1600-0765.2006.00924.x.
11. Sahrmann P, Winkler S, Gubler A, Attin T. Assessment of implant surface and instrument insert changes due to instrumentation with different tips for ultrasonic-driven debridement. BMC Oral Health. 2021;21(1):25. doi:10.1186/s12903-020-01384-0.
12. Keim D, Nickles K, Dannewitz B, Ratka C, Eickholz P, Petsos H. In vitro efficacy of three different implant surface decontamination methods in three different defect configurations. Clin Oral Implants Res. 2019;30(6):550–558. doi:10.1111/clr.13441.
13. Sahrmann P, Ronay V, Hofer D, Attin T, Jung RE, Schmidlin PR. In vitro cleaning potential of three different implant debridement methods. Clin Oral Implants Res. 2015;26(3):314–319. doi:10.1111/clr.12322.
14. Luengo F, Sanz-Esporrín J, Noguerol F, Sanz-Martín I, Sanz-Sánchez I, Sanz M. In vitro effect of different implant decontamination methods in three intraosseous defect configurations. Clin Oral Implants Res. 2022;33(11):1087–1097. doi:10.1111/clr.13991.
15. Sanz-Martín I, Paeng K, Park H, Cha JK, Jung UW, Sanz M. Significance of implant design on the efficacy of different peri-implantitis decontamination protocols. Clin Oral Investig. 2021;25(6):3589–3597. doi:10.1007/s00784-020-03681-y.
16. Park JB, Jang YJ, Koh M, Choi BK, Kim KK, Ko Y. In vitro analysis of the efficacy of ultrasonic scalers and a toothbrush for removing bacteria from resorbable blast material titanium disks. J Periodontol. 2013;84(8):1191–1198. doi:10.1902/jop.2012.120369.
17. Park JB, Lee SH, Kim N, Park S, Jin SH, Choi BK, et al. Instrumentation With Ultrasonic Scalers Facilitates Cleaning of the Sandblasted and Acid-Etched Titanium Implants. J Oral Implantol. 2015;41(4):419–428. doi:10.1563/aaid-joi-d-13-00078.
18. Schwarz F, Sculean A, Rothamel D, Schwenzer K, Georg T, Becker J. Clinical evaluation of an Er:YAG laser for nonsurgical treatment of peri-implantitis: a pilot study. Clin Oral Implants Res. 2005;16(1):44–52. doi:10.1111/j.1600-0501.2004.01051.x.
19. Toma S, Behets C, Brecx MC, Lasserre JF. In Vitro Comparison of the Efficacy of Peri-Implantitis Treatments on the Removal and Recolonization of Streptococcus gordonii Biofilm on Titanium Disks. Materials (Basel). 2018;11(12). doi:10.3390/ma11122484.
20. Toma S, Brecx MC, Lasserre JF. Clinical Evaluation of Three Surgical Modalities in the Treatment of Peri-Implantitis: A Randomized Controlled Clinical Trial. J Clin Med. 2019;8(7). doi:10.3390/jcm8070966.
21. Toma S, Lasserre J, Brecx MC, Nyssen-Behets C. In vitro evaluation of peri-implantitis treatment modalities on Saos-2osteoblasts. Clin Oral Implants Res. 2016;27(9):1085–1092. doi:10.1111/clr.12686.
22. Al-Hashedi AA, Laurenti M, Benhamou V, Tamimi F. Decontamination of titanium implants using physical methods. Clin Oral Implants Res. 2017;28(8):1013–1021. doi:10.1111/clr.12914.
23. Pérez-Tanoira R, Fernández-Arias M, Potel C, Carballo-Fernández R, Pérez-Castro S, Boutinguiza M, et al. Silver Nanoparticles Produced by Laser Ablation and Re-Irradiation Are Effective Preventing Peri-Implantitis Multispecies Biofilm Formation. Int J Mol Sci. 2022;23(19). doi:10.3390/ijms231912027.
24. Giannelli M, Landini G, Materassi F, Chellini F, Antonelli A, Tani A, et al. Effects of photodynamic laser and violet-blue led irradiation on Staphylococcus aureus biofilm and Escherichia

coli lipopolysaccharide attached to moderately rough titanium surface: in vitro study. Lasers Med Sci. 2017;32(4):857–864. doi:10.1007/s10103-017-2185-y.
25. Hiltunen AK, Savijoki K, Nyman TA, Miettinen I, Ihalainen P, Peltonen J, et al. Structural and Functional Dynamics of Staphylococcus aureus Biofilms and Biofilm Matrix Proteins on Different Clinical Materials. Microorganisms. 2019;7(12). doi:10.3390/microorganisms7120584.
26. Cha JK, Paeng K, Jung UW, Choi SH, Sanz M, Sanz-Martín I. The effect of five mechanical instrumentation protocols on implant surface topography and roughness: A scanning electron microscope and confocal laser scanning microscope analysis. Clin Oral Implants Res. 2019;30(6):578–587. doi:10.1111/clr.13446.
27. Kurt A, Cilingir A, Bilmenoglu C, Topcuoglu N, Kulekci G. Effect of different polishing techniques for composite resin materials on surface properties and bacterial biofilm formation. J Dent. 2019;90:103–199. doi:10.1016/j.jdent.2019.103199.
28. Reigada I, Pérez-Tanoira R, Patel JZ, Savijoki K, Yli-Kauhaluoma J, Kinnari TJ, et al. Strategies to Prevent Biofilm Infections on Biomaterials: Effect of Novel Naturally-Derived Biofilm Inhibitors on a Competitive Colonization Model of Titanium by Staphylococcus aureus and SaOS-2 Cells. Microorganisms. 2020;8(3). doi:10.3390/microorganisms8030345.
29. Romanos GE, Weitz D. Therapy of peri-implant diseases. Where is the evidence? J Evid Based Dent Pract. 2012;12(3 Suppl):204–208. doi:10.1016/s1532-3382(12)70038-6.
30. Louropoulou A, Slot DE, Van der Weijden F. The effects of mechanical instruments on contaminated titanium dental implant surfaces: a systematic review. Clin Oral Implants Res. 2014;25(10):1149–1160. doi:10.1111/clr.12224.
31. Schmage P, Thielemann J, Nergiz I, Scorziello TM, Pfeiffer P. Effects of 10 cleaning instruments on four different implant surfaces. Int J Oral Maxillofac Implants. 2012;27(2):308–317.
32. Renvert S, Samuelsson E, Lindahl C, Persson GR. Mechanical non-surgical treatment of peri-implantitis: a double-blind randomized longitudinal clinical study. I: clinical results. J Clin Periodontol. 2009;36(7):604–609. doi:10.1111/j.1600-051X.2009.01421.x.
33. Karring ES, Stavropoulos A, Ellegaard B, Karring T. Treatment of peri-implantitis by the Vector system. Clin Oral Implants Res. 2005;16(3):288–293. doi:10.1111/j.1600-0501.2005.01141.x.
34. Schmidt KE, Auschill TM, Heumann C, Frankenberger R, Eick S, Sculean A, et al. Influence of different instrumentation modalities on the surface characteristics and biofilm formation on dental implant neck, in vitro. Clin Oral Implants Res. 2017;28(4):483–490. doi:10.1111/clr.12823.
35. Baek SH, Shon WJ, Bae KS, Kum KY, Lee WC, Park YS. Evaluation of the safety and efficiency of novel metallic ultrasonic scaler tip on titanium surfaces. Clin Oral Implants Res. 2012;23(11):1269–1274. doi:10.1111/j.1600-0501.2011.02302.x.
36. Mann M, Parmar D, Walmsley AD, Lea SC. Effect of plastic-covered ultrasonic scalers on titanium implant surfaces. Clin Oral Implants Res. 2012;23(1):76–82. doi:10.1111/j.1600-0501.2011.02186.x.
37. Rühling A, Kocher T, Kreusch J, Plagmann HC. Treatment of subgingival implant surfaces with Teflon-coated sonic and ultrasonic scaler tips and various implant curettes. An in vitro study. Clin Oral Implants Res. 1994;5(1):19–29. doi:10.1034/j.1600-0501.1994.050103.x.
38. Hakki SS, Tatar G, Dundar N, Demiralp B. The effect of different cleaning methods on the surface and temperature of failed titanium implants: an in vitro study. Lasers Med Sci. 2017;32(3):563–571. doi:10.1007/s10103-017-2149-2.
39. Yang SM, Park JB, Ko Y. Use of confocal microscopy for quantification of plastic remnants on rough titanium after instrumentation and evaluation of efficacy of removal. Int J Oral Maxillofac Implants. 2015;30(3):519–525. doi:10.11607/jomi.3500.
40. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res. 2006;17 Suppl 2:68–81. doi:10.1111/j.1600-0501.2006.01353.x.