

Original Article

Antibiofilm Efficacies of Cold Plasma and Er:YAG Laser on *Staphylococcus aureus* Biofilm on Titanium for Nonsurgical Treatment of Peri-Implantitis

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INTRODUCTION

Over the past two decades, dental implants have revolutionized the treatment option for fully or partially edentulous patients. The use of implants has become a desired treatment approach due to their high predictability. Even though dental implants have been defined to succeed long-terms, failure may occur due to treatment planning, surgical, and prosthetic practice and maintenance.^[1,2] The inflammatory lesions that are developed in soft and hard tissues around dental implants are called peri-implantitis.^[3,4] The prevalence of peri-implantitis is reported approximately 11.3%–47.2% after implant therapy.^[5] Salivary glycoproteins adhere to titanium surfaces with accompanying microbiological colonization shortly after implant placement. The biofilm formation plays a critical role in onset and progression

ABSTRACT

Objectives: The aim of the present study was to compare antibiofilm efficacies of the laser in contact and noncontact application modes and cold atmospheric plasma (CAP) on *Staphylococcus aureus* biofilm grown on sandblasted, large grit, acid-etched (SLA) titanium discs as an *in vitro* model of biofilm eradication on dental implant materials. **Methods:** *S. aureus* biofilm was matured on titanium discs for 7 days then, treated with contact and noncontact Er:YAG laser and CAP. Antibiofilm efficacy of laser and plasma treatments were evaluated with colony counting and safranin assays. Surface characteristics of titanium disc were analyzed with scanning electron microscopy and surface roughness measurements. Temperature distribution over titanium discs were presented for the thermal safety assessment of laser and plasma treatments. **Results:** CAP resulted in 6-log inactivation of *S. aureus* biofilm, whereas biofilm inactivation was determined as 1 and 2.7-log for noncontact and contact laser treatments, respectively. Laser and plasma treatments did not cause any alterations on the roughness of titanium discs. Contact laser treatment caused a focal temperature increase up to 58°C, whereas plasma treatment led a uniform temperature distribution on the disc within safe limits. **Conclusion:** CAP showed superior antibiofilm activity on 7-day-old *S. aureus* biofilm grown over SLA titanium discs, compared to contact and noncontact laser treatment without temperature increase and any damage to the surface of titanium discs.

KEYWORDS: Cold atmospheric plasma, lasers, peri-implantitis, titanium discs

of the disease and is the main factor for the development of infection around implants.^[1]

Biofilm is defined as the cluster of microorganisms that adhere to a surface and each other.^[6] Biofilm formation process is triggered by adherence of microbial cells on a surface and followed by series of further steps involving secretion of extracellular polymeric substance (EPS) to provide structural integrity and enhance the nutrition of cells.^[7] Microorganisms are more resistant to antimicrobial agents in their biofilm

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forms compared to planktonic forms.^[8] The presence of *Staphylococcus aureus* and *Candida albicans* along with enteric rods are closely associated with peri-implantitis.^[9,10] The affinity of *S. aureus* to titanium surfaces to form biofilm has been shown in *in vitro* studies.^[11] In addition, *S. aureus* was reported to be a putative pathogen in the onset of peri-implantitis.^[9] Mechanical and/or chemical decontamination of exposed implant surfaces is indispensable for treatment of peri-implantitis.^[12] Mechanical peri-implant therapy *per se* might remain insufficient for complete eradication of pathogens due to their location and adhesion within the gingival and implant surfaces.^[13] Mechanical debridement of microstructured surfaces is more difficult and less effective for decontamination than that of smooth surfaces. Consequently, establishing a stable debridement procedure without alterations on surface microstructures is needed.^[12] Currently, novel methods such as lasers, cold atmospheric plasma (CAP), photodynamic therapy, and ozone (O₃) have been suggested for peri-implant therapy.^[13] The use of dental lasers has become popular for decontamination of implant surfaces. While lasers are reported as a perfect treatment option for decontamination of implant surfaces, they have drawbacks such as causing damage and alterations on implant surfaces.^[14] Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser causes melting effect on titanium surfaces. The erbium-doped yttrium aluminum garnet (Er:YAG) laser (2940 nm wavelength) has strongly been absorbed by water and is suitable for both hard and soft tissue management.^[12] Furthermore, Er:YAG lasers have been shown as efficient instruments for the treatment of peri-implantitis with their numerous advantages and bactericidal effects.^[15] Plasma is the fourth state of matter after solid, liquid, and gas and can be generated under electric field artificially.^[16] There are two types of plasma as follows: thermal and nonthermal or CAP.^[17] CAP is defined as an ionized gas whose temperature remains close to room temperature and includes a collection of charged particles, reactive species, and radicals that could readily react with biological materials and microorganisms, including bacteria, and eukaryotic cells and tissues as well.^[18] Decontamination procedures with CAP have emerged in the recent years and the antimicrobial effect of cold plasmas has been demonstrated for a variety of microorganisms.^[19-22]

Furthermore, a wide range of dental applications of cold plasma was reported thank to its compatible temperatures.^[21,23]

The hypotheses of the present study are;

- a. CAP is as efficient as Er:YAG laser for eradication of biofilm from titanium surfaces
- b. Contact and noncontact irradiation of laser is not different in terms of eradication of biofilm on titanium surfaces and altering the surface structures of titanium
- c. There are no differences among CAP and laser in altering the surface structures of titanium.

The aim of the present study was to compare antibiofilm efficacies of the laser in contact and noncontact application modes and CAP on *S. aureus* biofilm grown on sandblasted, large grit, acid-etched (SLA) titanium discs as an *in vitro* model of biofilm eradication on dental implant materials.

MATERIAL AND METHODS

Titanium discs

Seventy-six, 2 mm thick SLA titanium discs with 8 mm diameter (NucleOSS, İzmir, Turkey) were used in all experiment. Discs were sterilized with gamma irradiation by the manufacturer.

Biofilm formation on titanium discs

For the growth of bacterial biofilms on titanium discs, *S. aureus* ATCC 25923 reference strain was used. One mL frozen stocks of *S. aureus* that were kept in -80°C, were thawed at room temperature, transferred into 9 mL trypticase soy broth (TSB) and incubated in shaker incubator at 37°C and 120 rpm for overnight. Biofilm formation medium was prepared by adding 100 µl of 10^[8] colony forming units (CFU)/mL *S. aureus* suspension into 10 mL of TSB. Hundred microliter of 50% (w/v) glucose solution which yields 0.5% final concentration of glucose was added into biofilm formation medium to enhance bacterial adherence and biofilm growth.

Since, only upper surfaces of discs were intended to be treated with laser and plasma, biofilm growth were allowed only on the upper surfaces of titanium discs. For this purpose, parafilm stripes were cut according to the size of discs and wiped with 70% ethanol and held under UV for 1 h. Then, lower and lateral surfaces of titanium discs were wrapped with parafilm stripes to allow biofilm growth on the upper surface of titanium discs. Wrapped titanium discs were transferred into 24 well-plate, 1 mL of biofilm formation medium were added on discs and incubated in stationary incubator for 7 days, at 37°C. Medium on discs was refreshed with TSB that was supplied with glucose solution every other day.

After completion of 7 days of incubation, parafilm around discs were removed and discs were gently washed for twice using 1X sterile phosphate buffered saline (PBS) solution prior to plasma or laser treatment.

Plasma and laser treatments

Laser treatments of biofilms grown on titanium discs were performed using an Er:YAG laser system (Fotona, Twinlight AT Fileds, Ljubljana, Slovenia). The laser system was operated at 2490 nm wavelength, 100 mJ/pulse power and 10 pulse-per-second frequency for 30 s. A water-cooled, R02 handpiece and R14 handpiece were used for noncontact and contact mode laser treatments, respectively. CAP treatment of biofilms was performed with “plasma ONE” (Plasma Medical Systems, Bad Ems, Germany) device. Plasma ONE system was operated at 2.5 μ s of pulse width, 1.2 kHz frequency, and 5 W of power output for 2 min at a fixed 1 mm of discharge gap with PS12 probe [Figure 1].

Colony counting assay

Subsequent to plasma, contact and noncontact laser treatments of biofilms, discs were transferred in to microcentrifuge tubes, 1 mL of 1X PBS solution was added and discs were held in ultrasonic water bath for 15 min and then vortexed to remove living bacterial cells from the disc surface. Then, serial dilutions were made and bacteria were plated on trypticase soy agar (TSA) plates. TSA plates were incubated for 24 h at 37°C. Next day, surviving colonies were counted and expressed as log₁₀ surviving CFU. Untreated discs were used as control group. Seven discs were used in control and experimental groups.

Safranin assay

Amount of biofilm mass, including bacterial cells and EPS that is secreted by bacterial cells during formation of biofilm, was determined with safranin assay (F. İ). Following plasma, contact and noncontact laser treatments of biofilms, discs were transferred in to 24-well plate and washed twice using 1X PBS solution to remove nonadherent bacteria. Then, discs were kept inside biological safety cabin for 30 min to allow evaporation of excess liquid. A volume of 1 mL of 0.1% safranin was added on discs, held for 15 min and then removed. Discs were washed with 1X PBS to remove excess safranin and held in the biological safety cabin for drying. Then, 1 mL of 30% acetic acid was added on discs to dissolve safranin. Two hundred microliter of dissolved safranin dye was transferred to 96-well plate and absorbance was measured at 550 nm using multiwell plate spectrophotometer (Biotek Synergy HTX, Winooski, VT, USA). Untreated discs were used as control group. Average absorbance of control discs was normalized as 100% biofilm mass and biofilm mass amount on treated discs were expressed as percentage. Seven discs were used in control and experimental groups.

Scanning electron microscopy of samples

Biofilms on titanium discs were fixed to be performed for scanning electron microscope (SEM) imaging. After plasma, contact and noncontact lasers treatments, discs were held in 2.5% glutaraldehyde solution for 2 h, then rinsed with deionized water for once and transferred into 1% osmium tetroxide. Then, samples were rinsed again with deionized water and exposed serial ethanol baths to remove water inside cells and held in hexamethyldisilazane for 45 min and finally kept in desiccator for at least 24 h for drying. Imaging of samples was performed with SEM (JEOL JSM-6060, Tokyo, Japan) at 7 kV of voltage.

Temperature measurements

Temperature distribution on the surface of titanium discs was measured (T. P.) with thermal imaging camera immediately on completion of laser and plasma treatments (Testo 882, Hampshire, UK). Obtained images were analyzed with software provided with thermal imaging camera and histograms that show temperature distribution was plotted.

Surface roughness measurement

Following plasma, contact and noncontact laser treatments, surface roughness of titanium discs were measured (F. İ.) using a profilometer (Mitutoyo Surfest SJ-210, Kanagawa, Japan) equipped with 5- μ m diamond-tracing stylus tip. Five discs from each group were used and three random areas per each disc were measured over a scan length of 500 μ m and with a scanning rate of 100 μ m/s to obtain arithmetic mean roughness (Ra, μ m).

Statistical analysis

All variables were normally distributed and therefore, data were analyzed with parametric tests. Differences between groups were tested by one-way analysis of variance, followed by Tukey's honest significant difference test for pair-wise comparisons. The value of $P < 0.05$ was considered to be statistically significant for all analyses.

RESULTS

Antibiofilm activities of Er:YAG lasers and cold atmospheric plasma treatments

Colony counting assays showed 7.3-log CFU/mL of mean biofilm growth on control SLA titanium disc surfaces. Colony-counting assay results revealed more than 6-log eradication of *S. aureus* biofilm on SLA titanium disc surfaces in consequence of 120-s CAP treatment. Besides, 30-s Er:YAG laser treatment eradicated about 1 and 2.7-log of biofilm for noncontact and contact mode, respectively ($P < 0.05$) [Figure 2a]. Furthermore,

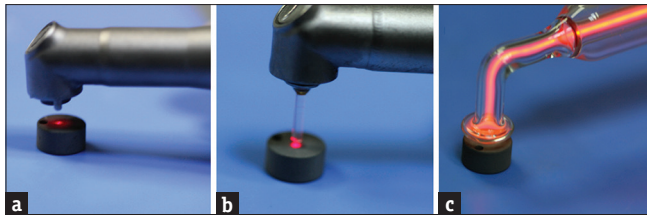


Figure 1: Noncontact Er:YAG laser treatment (a); there is a gap between surface of the laser hand piece and titanium disc. Indicator light of laser is spread over the surface of the titanium disc, suggests that noncontact laser effect is also spread over the disc. Contact Er:YAG laser treatment (b); tip of the laser hand piece touches to the surface of the disc and laser affects the point of contact and cold atmospheric plasma treatment (c); electrode covers the whole area of the titanium disc therefore hovering of plasma electrode is not necessary contrary to laser and uniform effect is most likely expected

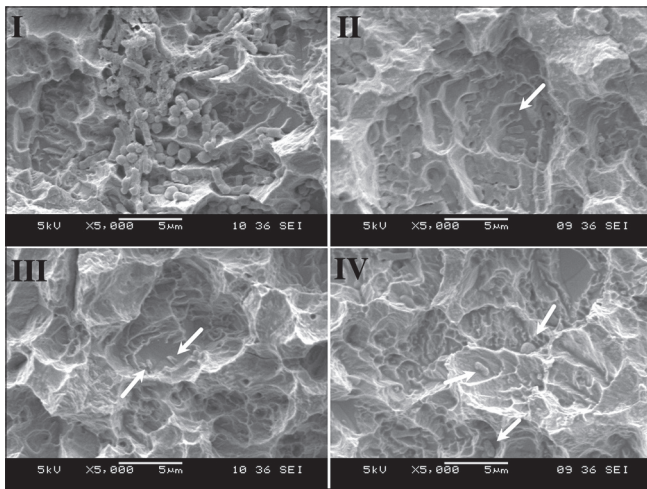


Figure 3: Scanning electron microscope images of titanium discs after noncontact and contact Er:YAG laser and cold atmospheric plasma treatments of titanium discs with 7-day-old *Staphylococcus aureus* biofilm growth to evaluate antibiofilm effect of treatment modalities ($\times 5000$). Growth of *Staphylococcus aureus* biofilm on control disc along with the presence of extracellular polymeric substance is clearly visible (I). Following treatment of biofilm-grown discs with noncontact and contact laser and cold atmospheric plasma, damaged and inactivated bacterial cells were observed (indicated with white arrows) (II, III, IV)

Table 1: Surface topographic properties of the SLA titanium discs (mean \pm standard deviation) after contact, non-contact and CAP treatment using surface profilometer

	Surface Roughness Control	Contact laser	Noncontact laser	Plasma	P
Ra (μm)	1.33 \pm 0.05	1.32 \pm 0.15	1.22 \pm 0.12	1.29 \pm 0.17	0.121

safranin assay results demonstrated that all treatment modalities led statistically significant and substantial level of reduction in biofilm mass [Figure 2b]. In detail, CAP, noncontact Er:YAG laser, and contact Er:YAG laser treatments caused about 62%, 47%, and 74% reduction in biofilm mass, respectively ($P < 0.05$). Moreover, SEM images showed consistent findings with colony counting

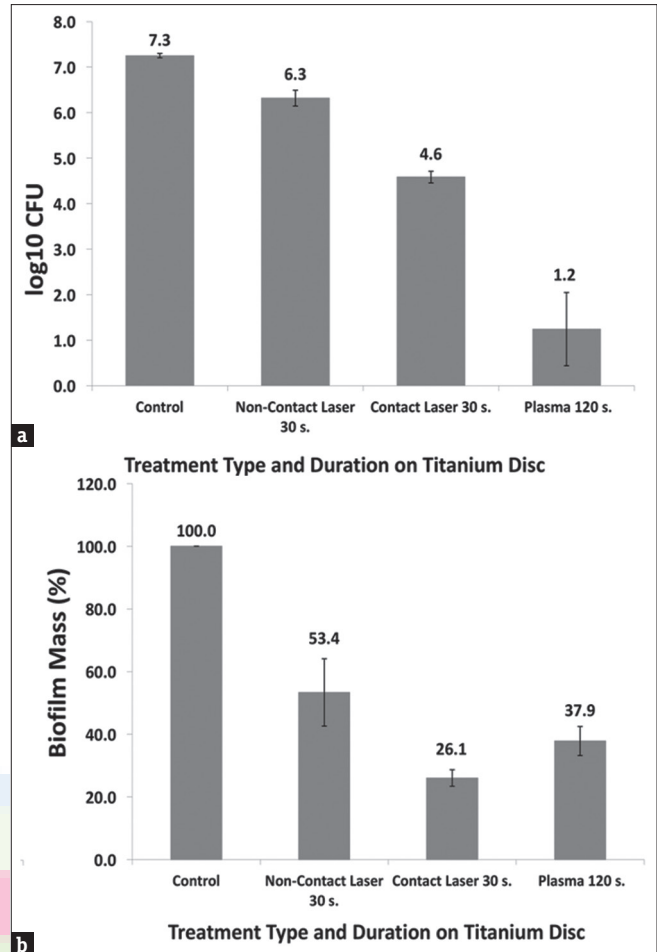


Figure 2: Colony counting assay results after noncontact and contact Er:YAG laser and cold atmospheric plasma treatments of titanium discs with 7-day-old *Staphylococcus aureus* biofilm growth. Cold atmospheric plasma leads more than 6-log inactivation while biofilm inactivation rate was determined around 1-log for noncontact laser and 2.7-log for contact laser treatment ($P < 0.05$) (a). Safranin assay results after noncontact and contact Er:YAG laser and cold atmospheric plasma treatments of titanium discs with 7-day-old *Staphylococcus aureus* biofilm growth. All treatment modalities resulted in statistically significant reduction in biofilm mass amount ($P < 0.05$) (b)

and safranin assays [Figure 3]. SEM images of control discs revealed EPS in the form of web-like structure and adherent *S. aureus* cells forming biofilm as represented in Figure 3-I. Furthermore, SEM images of Er:YAG and CAP treated discs showed damaged *S. aureus* cells on the surface of titanium Figure 3-II, III and IV (indicated with white arrows).

Surface analyses of titanium discs

SEM images presented [Figure 4] did not reveal any remarkable change on the surfaces of titanium discs following all treatment modalities. While 30 s of treatment with noncontact laser caused statistically nonsignificant reduction on surface roughness of SLA titanium discs, plasma, and contact laser treatments did not cause alteration on surface roughness [Table 1].

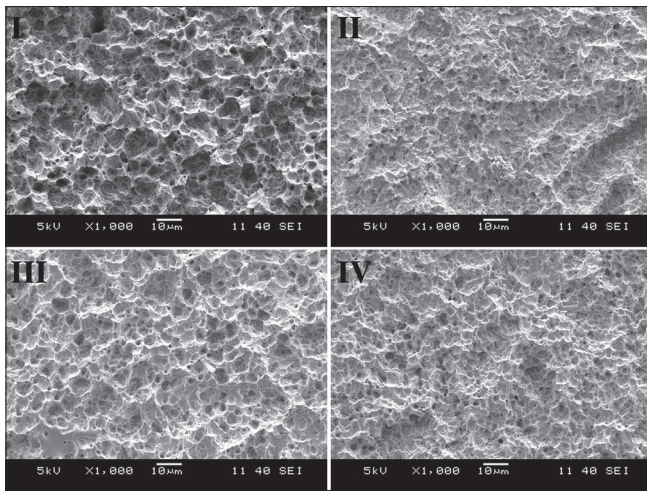


Figure 4: Without biofilm growth to evaluate effect of treatment modalities on titanium discs ($\times 1000$). Besides, any remarkable alteration on the surface morphology of titanium discs was not observed for any of treatment modalities in comparison with control disc (I, II, III, and IV). Note that $\times 1000$ magnified images were presented for evaluation of surface morphology to visualize a larger area

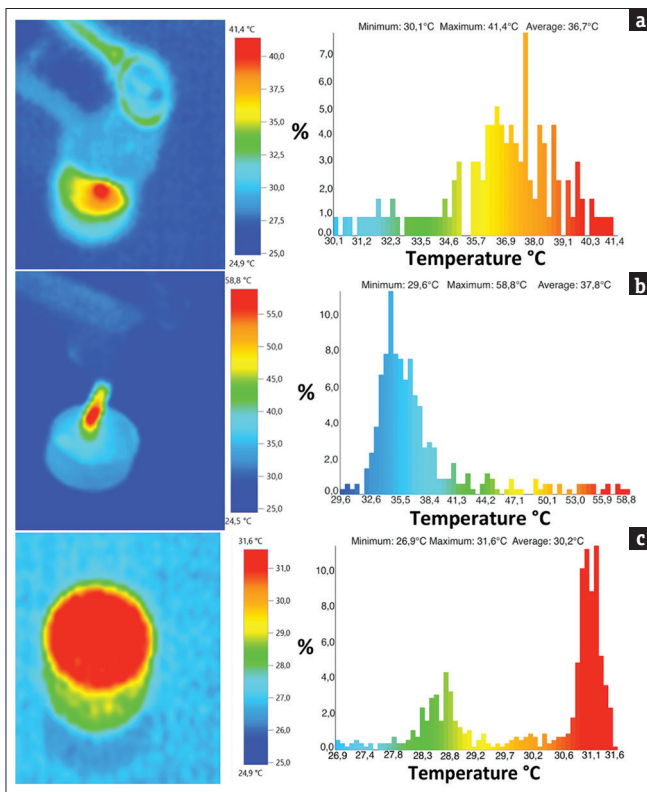


Figure 5: Temperature distribution on titanium disc surfaces immediately after non-contact (a), contact (b) Er:YAG laser and cold atmospheric plasma (c) treatment. Note that in contact laser treatment, thermal effect of contact laser remains in a narrower area at the point of contact (b) while thermal effect of noncontact laser was spread over the disc surface (a). Cold atmospheric plasma affected whole disc surface homogenously (c)

Temperature distribution on titanium discs

After plasma treatment, a homogenous temperature distribution was observed [Figure 5]. Maximum

and average temperatures on disc surface after plasma treatment were 31.6°C and 30.2°C , respectively [Figure 5a]. In noncontact laser treatment, thermal effect of the laser was spread over the surface of the titanium disc while in contact laser treatment temperature increase was limited on the contact point of the laser handpiece tip over the disc. In noncontact laser treatment, maximum temperature, and average temperatures were 41.4°C and 36.7°C , respectively [Figure 5b] while maximum temperature was 58.8°C and the average temperature was 37.8°C for contact laser treatment [Figure 5c].

DISCUSSION

Lasers have been in the scene of dentistry since the 1960s, the first introduction by Maiman.^[24] As part of dental applications, lasers have been used to eradicate microorganisms growing on dental implants for the treatment of peri-implantitis.^[14] The antibiofilm activity of lasers on bacterial and fungal biofilms for the treatment of peri-implantitis was widely studied and reported by various researchers.^[25-27]

Strong and broad-spectrum antibacterial activity of nonthermal atmospheric plasmas has taken attention of researchers and CAPs have been introduced as an alternative and novel tool for decontamination. Several researches have reported antibacterial activity of CAP on biofilms.^[21,28,29] Moreover, antibiofilm activity of CAP on biofilm that was grown on implant materials has been demonstrated. Duske *et al.* have tested antibiofilm activity of various CAP sources on *Staphylococcus epidermidis* grown on titanium (Ti6Al4V) discs and reported that CAP jet can inactivate more than 95% of *S. epidermidis* biofilm.^[30] Similarly, Ibis *et al.* have reported that dielectric barrier discharge plasma treatment of *S. aureus* and *E. coli* biofilms grown on titanium discs causes almost complete inactivation along with reduced EPS.^[28] Results demonstrated in the present study are consistent with findings from the literature. In brief, CAP inactivated more than 6-log of 7-day-old *S. aureus* biofilm grown on SLA titanium surface, whereas 1 and about 2.7-log inactivation was achieved with noncontact and contact laser application, respectively.

In addition to bacterial inactivation, safranin assay results demonstrated that laser and plasma treatment of contaminated titanium discs jeopardize the biofilm viability. In the present study, reduction of biofilm mass in consequence of noncontact, contact laser, and plasma treatments of contaminated titanium discs was demonstrated. The decrease in the amount of biofilm mass on titanium discs was statistically significant for noncontact, contact laser, and plasma treatments

compared to control group. Staining biofilms are useful for determination of total biofilm mass that includes EPS and bacterial cells. However, it does not fully reflect biofilm viability since EPS amount after an antimicrobial treatment has to be taken into consideration and be correlated with remaining viable bacteria.^[31] When an antimicrobial technique destroys the EPS, remaining viable bacteria are capable of secreting new EPS and sustain biofilm. On the other hand, an antimicrobial technique only kills bacteria, and then remaining EPS may serve a medium for recolonization.^[32] Moreover, especially on discs treated with CAP, crack-like damages on the cell surface were remarkable and consistent with previously reported studies.^[33]

Taken together, in the present study, CAP treatment provided a superior antibiofilm efficacy on 7-day-old *S. aureus* biofilm grown on SLA titanium surface compared to noncontact and contact laser treatments.

Even though the strong antibiofilm efficacy of CAP on 7-day-old *S. aureus* biofilm grown on SLA titanium discs was shown, the complexity of the biofilm in case of peri-implantitis should be considered. The structure of biofilm on the implant surfaces causing peri-implantitis is more complex and involves multispecies microorganisms including fungus.^[34] Therefore, further studies utilizing multispecies biofilm is needed to validate the potential use of CAP for nonsurgical treatment of peri-implantitis.

Both laser and CAP alter surface roughness of various dental implants.^[35-37] However, in the present study no significant alterations of the surface roughness of SLA titanium discs were observed after treatment with contact, noncontact Er:YAG laser and CAP treatments. In addition, on the SEM images, any visible changes in the surface morphology were not observed. The absence of a significant change of surface roughness could be attributed to lower doses of applied plasma and laser which are sufficient to inactivate bacteria into some extent while insufficient to affect surface properties of SLA titanium discs.

Antimicrobial efficacy of laser treatment is attributed to thermal effect, whereas in plasma treatment, this effect is mainly governed by the formation of reactive oxygen species and reactive nitrogen species.^[38,39] Moreover, atmospheric cold plasmas could be utilized for the treatment of heat-sensitive materials since temperature remains close to room temperature during atmospheric cold plasma application.^[17] In the present study, an increase in temperature was observed with laser treatments [Figure 4]. In noncontact laser treatment, the temperature reached up to 41.4°C and gradually reduced on the titanium disc surface. In contact laser treatment,

the rise in temperature was limited to the point of application where the laser's contact tip was in contact with titanium disc and a maximum 58.8°C temperature was measured. In a previous study, 47°C was reported as a threshold value for heat-induced thermal damage of the bone tissue, and temperature values above this value may trigger the bone necrosis.^[40] In the present study, after treatment of discs with plasma, temperature distribution was homogenous on the disc surface, and the maximum temperature was 31.6°C. Especially during contact laser treatment, the temperature may increase above the threshold temperature level which then may induce bone damage. As shown in the present study, during contact mode laser treatment, temperature increase was limited to an area where the laser tip was in contact with the titanium discs. However, in the actual clinical application, laser treatment is done within a narrower area due to the smaller size of dental implant compared to the size of titanium discs used in this study. Therefore, the contact laser should be used with caution in actual clinical practice to prevent thermal damage to the bone and surrounding tissue. Atmospheric cold plasma could be considered as a safer method compared to contact laser in terms of thermal damage to the bone and tissue surrounding tissue.

The limitation of the study could be different durations of laser and plasma treatments. In the present study, noncontact and contact lasers were applied for 30 s, whereas plasma treatment time was 120 s. The inconsistency of laser and plasma treatment durations may raise question for the comparison of antibiofilm activity. However, 30-s treatment time of laser application was used based on the literature^[12,26] and 120-s treatment time for plasma application was determined based on the suggestion of the manufacturer of the plasma one system. Thus, treatment durations are constraints of laser and plasma devices used in the present study. Furthermore, 120-s plasma treatment duration compared to 30-s laser treatment duration is thought to be considerably acceptable for clinical applications. The higher inactivation obtained by contact laser treatment compared to noncontact laser application was attributed to the mechanical removal of biofilm.

CONCLUSION

To the best of our knowledge, this is the first study to compare antibiofilm efficacies of contact, noncontact laser and CAP treatments for the management of peri-implantitis *in vitro*. The study results revealed that CAP treatment shows better antibiofilm activity compared to contact and noncontact laser treatment and also disrupts the biofilm integrity within the limitations

of this study. Moreover, CAP treatment does not change the roughness of SLA titanium surface. Furthermore, CAP treatment did not increase the temperature to the dangerous limits, which subsequently may trigger bone necrosis over the applied area. As a new technology, CAP could be considered as a novel tool for the management of peri-implantitis over laser treatment. However, further investigation, on animal models is required for a better understanding of the utilization of CAP for its possible applicability in clinical practice for the treatment of peri-implantitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Peri-implant mucositis and peri-implantitis: A current understanding of their diagnoses and clinical implications. *J Periodontol* 2013;84:436-43.
2. Papataniasiou E, Finkelman M, Hanley J, Parashis AO. Prevalence, etiology and treatment of peri-implant mucositis and peri-implantitis: A Survey of periodontists in the United States. *J Periodontol* 2016;87:493-501.
3. Zitzmann NU, Berglundh T. Definition and prevalence of peri-implant diseases. *J Clin Periodontol* 2008;35:286-91.
4. 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;26:937-41.
5. Koldslund OC, Scheie AA, Aass AM. Prevalence of peri-implantitis related to severity of the disease with different degrees of bone loss. *J Periodontol* 2010;81:231-8.
6. Chen M, Yu Q, Sun H. Novel strategies for the prevention and treatment of biofilm related infections. *Int J Mol Sci* 2013;14:18488-501.
7. Veerachamy S, Yarlagadda T, Manivasagam G, Yarlagadda PK. Bacterial adherence and biofilm formation on medical implants: A review. *Proc Inst Mech Eng H* 2014;228:1083-99.
8. Hota B. Contamination, disinfection, and cross-colonization: Are hospital surfaces reservoirs for nosocomial infection? *Clin Infect Dis* 2004;39:1182-9.
9. Heitz-Mayfield LJ, Lang NP. Comparative biology of chronic and aggressive periodontitis vs. Peri-implantitis. *Periodontol* 2000 2010;53:167-81.
10. Leonhardt A, Renvert S, Dahlén G. Microbial findings at failing implants. *Clin Oral Implants Res* 1999;10:339-45.
11. Harris LG, Mead L, Müller-Oberländer E, Richards RG. Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces. *J Biomed Mater Res A* 2006;78:50-8.
12. Taniguchi Y, Aoki A, Mizutani K, Takeuchi Y, Ichinose S, Takasaki AA, *et al.* Optimal Er:YAG laser irradiation parameters for debridement of microstructured fixture surfaces of titanium dental implants. *Lasers Med Sci* 2013;28:1057-68.
13. Yılmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T, *et al.* Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. *Photomed Laser Surg* 2013;31:293-8.
14. Tosun E, Tasar F, Strauss R, Kivanc DG, Ungor C. Comparative evaluation of antimicrobial effects of Er:YAG, diode, and CO2 lasers on titanium discs: An experimental study. *J Oral Maxillofac Surg* 2012;70:1064-9.
15. Ashnagar S, Nowzari H, Nokhbatolfighahaei H, Yaghoub Zadeh B, Chiniforush N, Choukhachi Zadeh N, *et al.* Laser treatment of peri-implantitis: A literature review. *J Lasers Med Sci* 2014;5:153-62.
16. Morfill GE, Kong MG, Zimmermann JL. Focus on plasma medicine. *New J Phys* 2009;11:115011-9.
17. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN and Fridman A. Applied plasma medicine. *Plasma Process Polym* 2008;5:503-33.
18. Kieft IE, van Berkel JJ, Kieft ER, Stoffels E. Radicals of plasma needle detected with fluorescent probe. In: *Plasma Process Polymers* (eds R. d'Agostino, P. Favia, C. Oehr and M. R. Wertheimer), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG; 2005. p. 293-308.
19. Sladek RE, Filoche SK, Sissons CH, Stoffels E. Treatment of *Streptococcus mutans* biofilms with a nonthermal atmospheric plasma. *Lett Appl Microbiol* 2007;45:318-23.
20. Ruf S, Lehmann A, Hannig M, Schäfer B, Schubert A, Feldmann U, *et al.* Killing of adherent oral microbes by a non-thermal atmospheric plasma jet. *J Med Microbiol* 2010;59:206-12.
21. Koban I, Holtfreter B, Hübner NO, Matthes R, Sietmann R, Kindel E, *et al.* Antimicrobial efficacy of non-thermal plasma in comparison to chlorhexidine against dental biofilms on titanium discs *in vitro* – Proof of principle experiment. *J Clin Periodontol* 2011;38:956-65.
22. Fricke K, Koban I, Tresp H, Jablonowski L, Schröder K, Kramer A, *et al.* Atmospheric pressure plasma: A high-performance tool for the efficient removal of biofilms. *PLoS One* 2012;7:e42539.
23. Foest R, Schmidt M, Becker K. Microplasmas, an emerging field of low-temperature plasma science and technology. *Int J Mass Spectrom* 2006;248:87-102.
24. Verma SK, Maheshwari S, Singh RK, Chaudhari PK. Laser in dentistry: An innovative tool in modern dental practice. *Natl J Maxillofac Surg* 2012;3:124-32.
25. Hauser-Gerspach I, Mauth C, Waltimo T, Meyer J, Stübinger S. Effects of Er:YAG laser on bacteria associated with titanium surfaces and cellular response *in vitro*. *Lasers Med Sci* 2014;29:1329-37.
26. Kreisler M, Kohnen W, Marinello C, Götz H, Duschner H, Jansen B, *et al.* Bactericidal effect of the Er:YAG laser on dental implant surfaces: An *in vitro* study. *J Periodontol* 2002;73:1292-8.
27. Sennhenn-Kirchner S, Schwarz P, Schliephake H, Konietzschke F, Brunner E, Borg-von Zepelin M, *et al.* Decontamination efficacy of erbium: yttrium-aluminium-garnet and diode laser light on oral *Candida albicans* isolates of a 5-day *in vitro* biofilm model. *Lasers Med Sci* 2009;24:313-20.
28. Ibis F, Oflaz H, Ercan UK. Biofilm inactivation and prevention on common implant material surfaces by nonthermal. *DBD Plasma Treat* 2016;6:33-45.
29. Idlibi AN, Al-Marrawi F, Hannig M, Lehmann A, Rueppell A, Schindler A, *et al.* Destruction of oral biofilms formed *in situ* on machined titanium (Ti) surfaces by cold atmospheric plasma. *Biofouling* 2013;29:369-79.
30. Duske K, Wegner K, Donnert M, Kunert U, Podbielski A, Kreikemeyer B. Comparative *in vitro* study of different atmospheric pressure plasma jets concerning their antimicrobial

- potential and cellular reaction. *Plasma Process Polym* 2015;12:1050-60.
31. Welch K, Cai Y, Strømme M. A method for quantitative determination of biofilm viability. *J Funct Biomater* 2012;3:418-31.
 32. Toté K, Berghe DV, Deschacht M, de Wit K, Maes L, Cos P, *et al.* Inhibitory efficacy of various antibiotics on matrix and viable mass of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Int J Antimicrob Agents* 2009;33:525-31.
 33. Poor AE, Ercan UK, Yost A, Brooks AD, Joshi SG. Control of multi-drug-resistant pathogens with non-thermal-plasma-treated alginate wound dressing. *Surg Infect (Larchmt)* 2014;15:233-43.
 34. Dhir S. Biofilm and dental implant: The microbial link. *J Indian Soc Periodontol* 2013;17:5-11.
 35. Faeda RS, Tavares HS, Sartori R, Guastaldi AC, Marcantonio E Jr. Evaluation of titanium implants with surface modification by laser beam. *Biomechanical study in rabbit tibias. Braz Oral Res* 2009;23:137-43.
 36. Seker E, Kilicarslan MA, Deniz ST, Mumcu E, Ozkan P. Effect of atmospheric plasma versus conventional surface treatments on the adhesion capability between self-adhesive resin cement and titanium surface. *J Adv Prosthodont* 2015;7:249-56.
 37. Vechiato-Filho AJ, da Silva Vieira Marques I, Dos Santos DM, Matos AO, Rangel EC, da Cruz NC, *et al.* Effect of nonthermal plasma treatment on surface chemistry of commercially-pure titanium and shear bond strength to autopolymerizing acrylic resin. *Mater Sci Eng C Mater Biol Appl* 2016;60:37-44.
 38. Lee BS, Lin YW, Chia JS, Hsieh TT, Chen MH, Lin CP, *et al.* Bactericidal effects of diode laser on *Streptococcus mutans* after irradiation through different thickness of dentin. *Lasers Surg Med* 2006;38:62-9.
 39. Ercan UK, Smith J, Ji HF, Brooks AD, Joshi SG. Chemical changes in nonthermal plasma-treated N-acetylcysteine (NAC) solution and their contribution to bacterial inactivation. *Sci Rep* 2016;6:20365.
 40. Eriksson AR, Albrektsson T. Temperature threshold levels for heat-induced bone tissue injury: A vital-microscopic study in the rabbit. *J Prosthet Dent* 1983;50:101-7.

