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Jiaying Zhu
First Affiliated Hospital of Dalian Medical University
Chunhui Zhao
First Affiliated Hospital of Dalian Medical University
Xixin Leng
First Affiliated Hospital of Dalian Medical University
Junyao Wan
First Affiliated Hospital of Dalian Medical University
Qiuhao Wang
First Affiliated Hospital of Dalian Medical University
Zhuoran Liang
Dalian Stomatological Hospital (Dalian University Affiliated Stomatological Hospital), Dalian 116011, China
Shengji Cao
Dalian Stomatological Hospital (Dalian University Affiliated Stomatological Hospital), Dalian 116011, China
Xiaoguang Xiao
Dalian Stomatological Hospital (Dalian University Affiliated Stomatological Hospital), Dalian 116011, China
Jianfeng Chen
chenjf123456780163.com

First Affiliated Hospital of Dalian Medical University

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Inhibitory Effect of Argon Cold Atmospheric Plasma on *Streptococcus mutans* Adhesion and Growth on Zirconia Surfaces

Jiaying Zhu[1]†, Chunhui Zhao[1]†, Xixin Leng[1], Junyao Wan[1], Qiuhao Wang[1], Zhuoran Liang[3], Shengji Cao[2]* , Xiaoguang Xiao[2]* , Jianfeng Chen[1]*

[1] Department of Stomatology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China

[2] Department of Clinical Laboratory, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China

[3] Dalian Stomatological Hospital (Dalian University Affiliated Stomatological Hospital), Dalian 116011, China

† Jiaying Zhu and Chunhui Zhao contributed equally to this work and should be considered co-first authors.

* Shengji Cao, Xiaoguang Xiao and Jianfeng Chen contributed equally to this work and should be considered co-corresponding authors.

Corresponding authors:

Jianfeng Chen, Department of Stomatology, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China; E-mail: chenjf12345678@163.com; Tel.: +86 18098876617

Shengji Cao, Department of Clinical Laboratory, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China; E-mail: jicharles@126.com; Tel.: +86 18098877288

Xiaoguang Xiao, Department of Clinical Laboratory, The First Affiliated Hospital of Dalian Medical University, Dalian 116011, China; E-mail: ckxxg@sina.com; Tel.: +86 13354096677

Author e-mail addresses: Jiaying Zhu: 2676813218@qq.com; Chunhui Zhao: zhaochunh2000@126.com; Xixin Leng: 502327345@qq.com; Junyao Wan: 748843176@qq.com; Qiuhao Wang: 786774417@qq.com; Zhuoran Liang: 2276772154@qq.com

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Abstract

Objectives To evaluate the inhibitory effect of argon cold atmospheric plasma (CAP) on *Streptococcus mutans* adhesion and growth on zirconia and its duration.

Materials and Methods Ninety zirconia specimens were divided into an immediate testing set (n = 24; control and CAP 30-, 60-, and 90-s groups) and a durability set (n = 66; CAP 60 s; T0–day 10); surface morphology and wettability were evaluated by SEM and contact-angle analysis. Specimens were inoculated with *S. mutans* (1.0×10^8 CFU/mL) and incubated at 37 °C in 5% CO_2 for 24 h; adhesion, viability, and metabolic activity were assessed by SEM, live/dead staining, and MTT assay.

Results CAP increased hydrophilicity without altering morphology; contact angles decreased from 97.09° ± 1.26° to 55.21°, 33.72°, and 20.01° in the 30-, 60-, and 90-s groups ($P < 0.05$). Treated surfaces showed fewer, more dispersed bacteria. MTT OD decreased from 0.62 ± 0.01 to 0.22 ± 0.02 (90-s group; $P < 0.05$). The antibacterial effect persisted for 2 days, then gradually declined to control levels by day 10.

Conclusions CAP effectively inhibits *S. mutans* adhesion and growth on zirconia; the immediate effect increased with treatment time (90 s strongest). A 60-s treatment provided a stable antibacterial window of about 2 days, lasting up to about 10 days.

Clinical Relevance Pretreating zirconia abutments with CAP may reduce early bacterial colonization during the critical peri-implant healing phase, promoting soft tissue sealing and long-term implant success.

Keywords: Cold atmospheric plasma; Zirconia; Implant abutment; *Streptococcus mutans*; Antibacterial activity

Introduction

Dental implants have a 10-year survival rate exceeding 95%, but their long-term success is still limited by biological complications [1]. The soft tissue seal around implant abutments acts as a barrier against oral bacterial invasion and is essential for long-term implant success [2, 3]. Peri-implant diseases are directly associated with biofilms formed by pathogenic bacteria [4]. *Streptococcus mutans*, an early colonizer of oral plaque biofilm, can adhere to implant surfaces at an early stage and participate in biofilm formation [5].

Yttria-stabilized tetragonal zirconia polycrystal (Y-TZP) is increasingly used for esthetic abutments because of its favorable aesthetics, biocompatibility, and mechanical strength [6]; however, its inherently bioinert surface may not be conducive to soft tissue adhesion and integration, increasing the risk of microbial invasion [7].

Cold atmospheric plasma (CAP), which is characterized by low temperature, high reactivity, and minimal tissue damage, has shown promise in biomedical applications. It reduces bacterial adhesion and biofilm formation on titanium surfaces [8] and promotes the adhesion and proliferation of host tissue cells [9, 10]. Nevertheless, studies on plasma application to zirconia remain scarce. This study aimed to evaluate the antibacterial effect of CAP treatment on zirconia surfaces and to explore its potential for promoting peri-implant soft tissue sealing.

Materials and Methods

Main materials and instruments

The standard *S. mutans* strain (ATCC 25175) was obtained from the Shanghai Bioresource Collection Center (Shanghai, China). Yttria-stabilized zirconia ceramic blocks were obtained from Shenzhen Upcera Dental Technology Co., Ltd. (Shenzhen, China). A dental restorative plasma activation device (model JIHY-PLASMA-20220915-3000; atmospheric-pressure argon plasma jet) was obtained from Changshu Jiujia Hengyi Biotechnology Co., Ltd. (Changshu, China). A ZEISS Sigma 360 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany), a Leica STELLARIS 5 confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany), and a KRUSS DSA100 contact angle goniometer (KRUSS GmbH, Hamburg, Germany) were used. SYTO 9 and propidium iodide (PI) stains were obtained from Invitrogen Corporation (Carlsbad, CA, USA), and MTT reagent was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Specimen preparation and grouping

Zirconia specimens were fabricated by CAD/CAM milling, sintered at 1550 °C, ground and polished to 6 mm × 6 mm × 2 mm, wet-ground with silicon carbide paper in successively finer steps up to 2000 grit, ultrasonically cleaned for 30 min, and UV-sterilized. A total of 90 specimens were prepared. Twenty-four specimens were randomly divided into four groups (n = 6 each): Group A (untreated control), Group B (CAP 30 s), Group C (CAP 60 s), and Group D (CAP 90 s) for immediate antibacterial testing. The remaining 66 specimens were all treated with CAP for 60 s and stored in sterile dishes at room temperature (25 °C) in the dark. Six specimens were assessed immediately after treatment (T0) and on days 1–10 for time-course testing. CAP parameters were as follows: high-purity argon as the working gas, a power of 0.5 kW, a gas pressure of 0.4 MPa, and a nozzle-to-specimen distance of 1.0 cm.

Surface characterization

(1) Surface morphology: the surface microstructure of specimens in each group was observed by SEM. (2) Surface wettability: the contact angle was measured using a contact angle goniometer. A 3-μL droplet of deionized water was placed on the specimen surface, and the mean of the left and right contact angles was calculated (six specimens per group).

Bacterial culture and inoculation

S. mutans was revived and cultured on Columbia blood agar at 37 °C in 5% CO_2 for 24–48 h and subcultured three times. Bacteria in the mid-exponential phase were inoculated into BHI broth and cultured with shaking at 180 rpm at 37 °C for 20 h. After centrifugation (5000 rpm, 4 °C, 5 min) and three washes with PBS, the suspension was adjusted to a 0.5 McFarland standard (1.0×10^8 CFU/mL) using a turbidimeter. Specimens were placed in 24-well plates, 2 mL of bacterial suspension was added to each well, and the plates were incubated at 37 °C in 5% CO_2 for 24 h.

Bacterial evaluation methods

(1) SEM observation: specimens were fixed in 2.5% glutaraldehyde at 4 °C overnight, washed with PBS, dehydrated through a graded ethanol series (30%, 60%, 90%, 95%, and 100%), air-dried, sputter-coated with gold, and observed by SEM. (2) Live/dead staining: SYTO 9 and PI were prepared at a ratio of 1.5:1000 and incubated with the specimens at 37 °C in the dark for 15 min. Images were acquired with a confocal laser scanning microscope (488 nm excitation, green = live bacteria; 561 nm excitation, red = dead bacteria). (3) MTT assay: after three washes with PBS, specimens were placed flat in 96-well plates. Twenty microliters of MTT solution (5 mg/mL) and 80 μL of BHI broth were added to each well, and the plates were incubated at 37 °C in 5% CO_2 in the dark for 4 h. The reaction solution was aspirated, 150 μL of dimethyl sulfoxide (DMSO) was added to each well, and the plates were shaken at room temperature for

15 min to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm with a microplate reader.

Statistical analysis

Statistical analysis was performed using SPSS 27.0 software (IBM Corp., Armonk, NY, USA). Quantitative data are presented as mean ± standard deviation (SD). Normality was assessed with the Shapiro–Wilk test, and homogeneity of variance was assessed with Levene's test. For comparisons among the four treatment groups, one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc test was used when variances were homogeneous. $P < 0.05$ was considered statistically significant.

Results

Surface morphology

SEM images of the zirconia surfaces are shown in Fig. 1a, b. Shallow striated scratches from wet grinding with silicon carbide paper were visible on the surfaces of Groups A and D. The surfaces were smooth without obvious cracks or depressions, and no notable differences in surface morphology were observed among the groups, indicating that CAP treatment did not alter the surface morphology of zirconia.

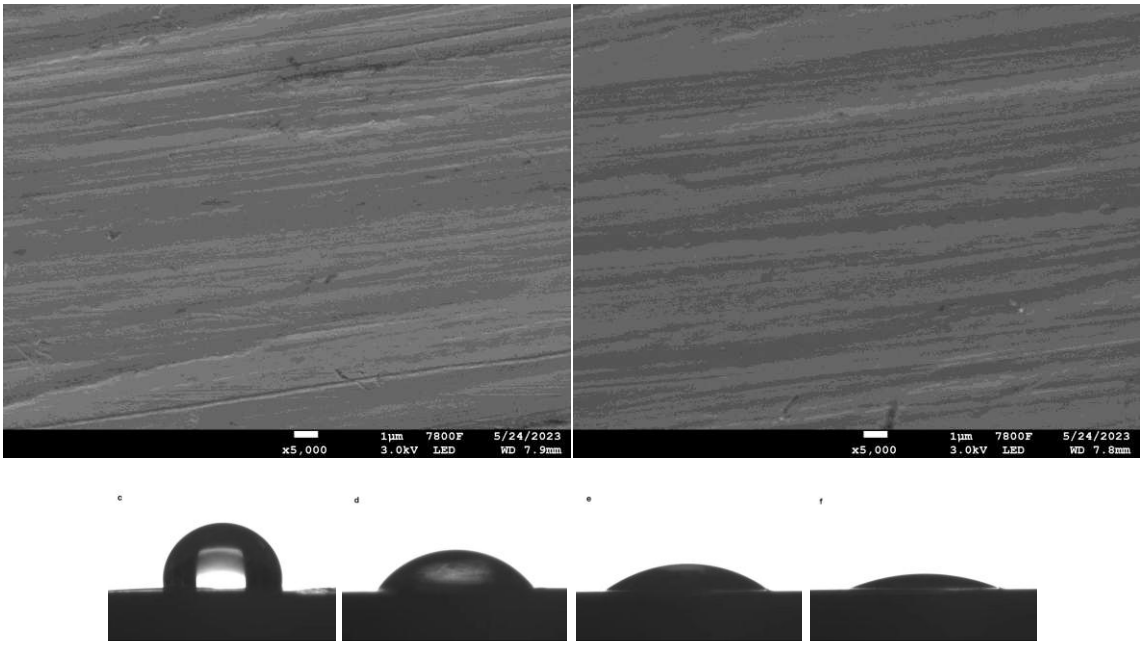


Fig. 1 Surface characterization of zirconia specimens. a, b SEM images of the untreated control (a) and CAP 90 s (b) surfaces (×5000; scale bars: 2 μm). c–f Representative water contact-angle images of the control (c), CAP 30 s (d), CAP 60 s (e) and CAP 90 s (f) groups

Surface wettability

The contact angle measurements are shown in Fig. 1c–f and Table 1. The contact angle of Group A was 97.09° ± 1.26°, and it decreased progressively in Groups B, C, and D. Surface hydrophilicity increased significantly with increasing CAP treatment time, and the differences among all groups were statistically significant ($P < 0.05$).

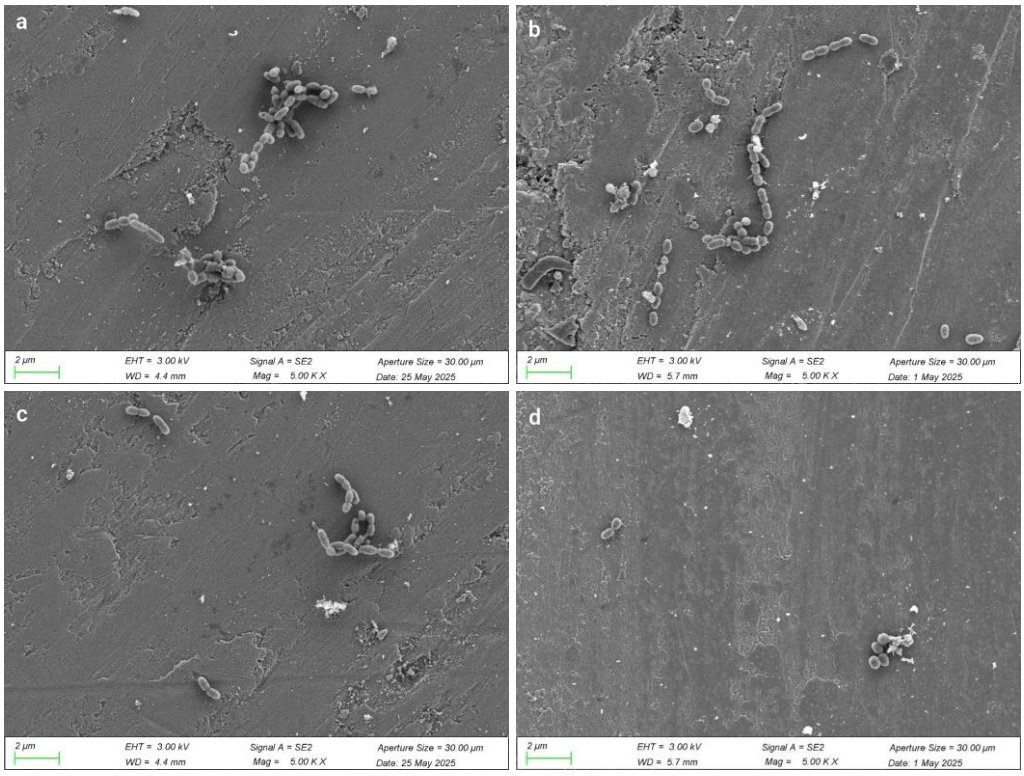
Table 1 Contact angles of zirconia surfaces in each group (°, mean ± SD)

Group	n	Contact angle (°, mean ± SD)
A	6	97.09 ± 1.26a
B	6	55.21 ± 1.98b
C	6	33.72 ± 2.78c
D	6	20.01 ± 2.24d

Different superscript letters indicate statistically significant differences ($P < 0.05$)

Bacterial morphology

SEM images of adherent bacteria are shown in Fig. 2a–d. In Group A, bacteria formed dense, intact chain-like aggregates with plump morphology. With increasing CAP treatment time, the number of adherent bacteria in Groups B, C, and D decreased progressively, their distribution became more dispersed, and the bacteria appeared shrunken and collapsed. In Group D, only a few bacteria with incomplete morphology were observed.



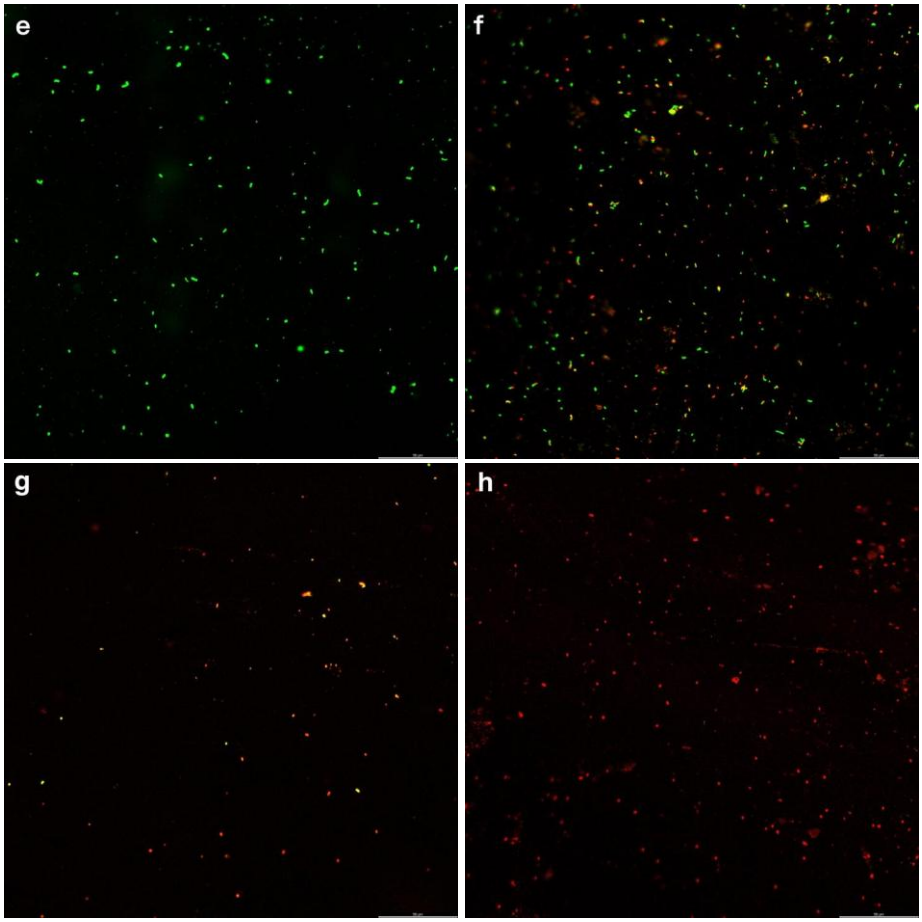


Fig. 2 Adhesion and viability of *S. mutans* on zirconia surfaces. a–d SEM images (×5000; scale bars: 2 μm) of *S. mutans* adherent to untreated control (a), CAP 30 s (b), CAP 60 s (c) and CAP 90 s (d) specimens. e–h Confocal laser scanning microscopy live/dead staining images of the corresponding groups (green, live bacteria; red, dead bacteria)

Bacterial viability (live/dead staining)

The live/dead staining results are shown in Fig. 2e–h. Group A showed dense green fluorescence, indicating predominantly live bacteria. With increasing CAP treatment time, green fluorescence gradually weakened and red fluorescence gradually intensified. Red fluorescence dominated in Group D, indicating markedly reduced bacterial viability and a significantly increased proportion of dead bacteria.

Bacterial metabolic activity (MTT assay)

The MTT results are shown in Fig. 3 and Table 2. The OD value of Group A was 0.62 ± 0.01, and it decreased progressively in Groups B, C, and D (0.41 ± 0.03, 0.38 ± 0.01, and 0.22 ± 0.02, respectively), with statistically significant differences among all groups ($P < 0.05$). The reduction in OD increased with CAP treatment time.

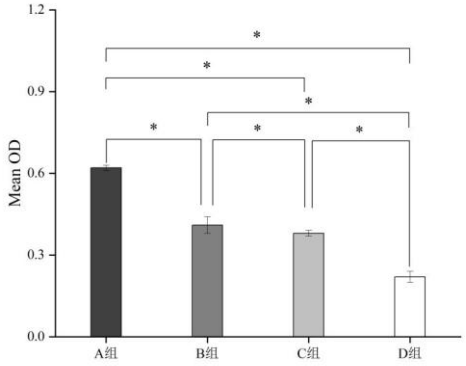


Fig. 3 MTT assay results of the four groups (n = 6; values are mean ± SD). Different letters indicate statistically significant differences ($P < 0.05$)

Table 2 MTT assay results of each group (OD value, mean ± SD)

Group	n	OD value (mean ± SD)
A	6	0.62 ± 0.01^{a}
B	6	0.41 ± 0.03^{b}
C	6	0.38 ± 0.01^{c}
D	6	0.22 ± 0.02^{d}

Different superscript letters indicate statistically significant differences ($P < 0.05$)

Time-dependent antibacterial effect (MTT assay)

The MTT results are shown in Fig. 4. For specimens treated with CAP for 60 s, the OD values from immediately after treatment (T0) to day 2 (T0, day 1, and day 2) were significantly lower than those of the control group (Group A) ($P < 0.05$), with no significant differences among these time points ($P > 0.05$), indicating a stable antibacterial effect. From day 3 onward, the OD values gradually increased, and by day 10 no significant difference from the control group was observed ($P > 0.05$), indicating that the antibacterial effect had essentially returned to control levels.

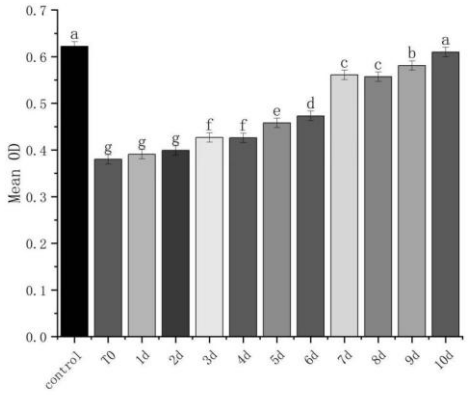


Fig. 4 Time-dependent antibacterial effect of 60 s CAP treatment assessed by the MTT assay (n = 6; values are mean ± SD). Identical letters indicate no statistically significant difference ($P > 0.05$); different letters indicate statistically significant differences ($P < 0.05$)

Discussion

Compared with conventional metal abutments, zirconia abutments provide excellent esthetic outcomes owing to their favorable biocompatibility, high mechanical strength, and translucency close to that of natural teeth [6, 11]. Despite these advantages, the inherently bioinert zirconia surface may not be conducive to the adhesion and integration of peri-implant soft tissue [6]. A stable soft tissue seal is the cornerstone of long-term esthetic outcomes and implant health; an inadequate seal directly increases the risk of microbial invasion, potentially leading to peri-implant mucositis and even peri-implantitis and jeopardizing long-term stability [2, 3]. Among surface modification approaches for dental implant materials, plasma surface treatment is one of the most widely studied. Figueira et al. [12] confirmed that cold atmospheric plasma has a marked bactericidal effect on oral biofilms, and Park et al. [13] reported that argon plasma treatment significantly increases the surface energy of zirconia and effectively reduces the adhesion of oral streptococci. Because argon has the advantages of low cost, low ionization energy, and effective surface cleaning, argon plasma was used in the present study.

SEM observation showed that CAP treatment for 90 s did not alter the surface morphology of zirconia, which is consistent with previous study [14]. The contact angle reflects the surface energy of a material: the greater the surface energy, the greater the hydrophilicity. In the present study, Group A exhibited the largest contact angle, and the contact angle of Group B decreased compared with that of the untreated group, indicating that CAP treatment enhanced the hydrophilicity of the zirconia surface. The contact angles of Groups C and D decreased markedly, indicating that longer CAP treatment resulted in significantly greater surface energy and hydrophilicity. These findings are consistent with those of Park et al. [13] and Henningsen et al. [14]. SEM observation of the bacteria–material interface clearly showed that the zirconia surface of Group A was densely covered with abundant *S. mutans* with intact morphology. With increasing CAP treatment time (Groups B to D), the number of adherent bacteria decreased markedly, and the bacteria on plasma-treated surfaces were more widely dispersed than those on untreated surfaces, indicating that CAP treatment inhibited bacterial colonization. These results are highly consistent with the findings of Yang et al. [15].

Confocal laser scanning microscopy clearly showed dense green fluorescence on the zirconia surface of the untreated control group, indicating that the surface was covered with numerous viable bacteria with intact membranes. With increasing CAP treatment time, the intensity and area of red fluorescence increased

significantly, whereas green fluorescence decreased correspondingly. When the treatment time was extended to 90 s, the proportion of dead (red) bacteria further increased. These results indicate that CAP treatment significantly reduced bacterial viability, and longer treatment resulted in greater inactivation, which is supported by the findings of Park et al. [13] and Yang et al. [16].

Most surface modification methods alter both surface topography and surface chemistry, and the resulting uncertainty regarding long-term biological effects limits their clinical translation. In the present study, the surface morphology of zirconia remained unchanged after CAP treatment, suggesting promise for clinical application. Human gingival fibroblasts and epithelial cells preferentially adhere to hydrophilic smooth surfaces, which may favor the formation of the peri-implant soft tissue seal [17]. However, increased surface hydrophilicity may also increase the risk of bacterial adhesion while promoting tissue integration, because the adhesion of oral pathogens is influenced by the surface energy and hydrophilicity of materials, and some species preferentially colonize hydrophilic interfaces [18]. In the present study, bacterial adhesion did not increase on the hydrophilic zirconia surfaces after CAP treatment, consistent with previous reports [15]. The underlying mechanism may be that the reactive oxygen and nitrogen species (RONS) generated by plasma persist on the material surface, directly killing bacteria and inhibiting their growth, thereby counteracting the proadhesive effect of hydrophilic surfaces [19]; however, this proposed mechanism needs to be verified by measuring the residual amount and types of RONS on material surfaces.

As a standard method for assessing cellular metabolic activity, the MTT assay showed that the OD values of all plasma-treated groups were significantly lower than that of the control group, indicating that CAP-treated zirconia surfaces effectively reduce bacterial metabolic activity, with the inhibitory effect increasing with treatment time. These results are consistent with previous reports [15, 16]. The present study showed that the antibacterial effect of 60-s CAP treatment remained stable from immediately after treatment to day 2 and then gradually declined, with bacterial metabolic activity returning to control levels by day 10. This time-dependent profile is closely related to the retention and decay of RONS on the material surface [16]. Yang et al. [16] demonstrated that large amounts of RONS can be retained on zirconia surfaces after helium plasma treatment; these reactive species exert antibacterial effects by oxidizing bacterial cell membranes, disrupting protein structure, and interfering with DNA replication, but their concentration gradually decreases over time. Park et al. [13] reported that the antibacterial effect of argon plasma-treated zirconia lasted approximately 48 h, which is largely consistent with the stable antibacterial effect within 2 days observed in the present study. Although the antibacterial effect declined over time, bacterial metabolic activity in the treated group remained lower than that in the control group during the intervening period (approximately days 3–9), suggesting a residual antibacterial effect; this is consistent with the conclusion of Yang et al. [16] that the treated group still retained antibacterial activity at 14 days. The stable antibacterial window in the early post-implantation phase (approximately 2 days) coincides with the critical period during which bacterial colonization on implant abutment surfaces is most likely to occur [20] and may provide a valuable window for soft tissue healing. Clinically, it is recommended that zirconia abutments be treated with plasma immediately before abutment placement to take full advantage of this optimal antibacterial time window.

Argon was selected as the working gas, and treatment times of 30, 60, and 90 s were chosen based on the following rationale: 30 s is the minimum effective time reported by Figueira et al. [12]; 60 s is the optimal time for promoting gingival fibroblast adhesion and proliferation reported by Zheng et al. [10]; and 90 s is the relatively effective antibacterial time reported by Yang et al. [15]. The present results suggest that 90-s treatment produces the strongest antibacterial effect; however, the effect of this treatment time on soft tissue cell behavior needs further investigation to determine the optimal parameters that balance antibacterial activity and soft tissue integration.

This study has several limitations. First, *S. mutans* was used, whereas the oral environment contains multispecies biofilms that may respond differently to CAP-treated zirconia. Second, all experiments were performed in vitro; the responses of soft tissue cells to CAP-treated surfaces, particularly at the antibacterial-optimal 90-s treatment, remain to be clarified. Third, the types and residual amounts of RONS on the treated surface were not quantified; future studies should correlate RONS decay with the time-dependent antibacterial profile. Finally, strategies to extend the duration of antibacterial activity, such as optimized gas mixtures or post-treatment coatings, warrant investigation.

Conclusions

CAP treatment significantly increases the hydrophilicity of zirconia surfaces and effectively inhibits *S. mutans* adhesion and growth without altering surface morphology. The immediate antibacterial effect increased with treatment time, with 90 s producing the strongest effect. The antibacterial effect of 60-s CAP treatment remained stable for approximately 2 days and then gradually declined, with an overall duration of up to approximately 10 days. This study provides in vitro evidence supporting the use of plasma treatment on zirconia implant surfaces; however, in vivo soft tissue integration, optimal treatment parameters, and strategies for extending the antibacterial effect require further investigation.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Not applicable.

Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Lee CT, Huang YW, Zhu L, Weltman R (2017) Prevalences of peri-implantitis and peri-implant mucositis: systematic review and meta-analysis. J Dent 62:1–12. https://doi.org/10.1016/j.jdent.2017.04.011

2. Ivanovski S, Lee R (2018) Comparison of peri-implant and periodontal marginal soft tissues in health and disease. Periodontol 2000 76:116–130. https://doi.org/10.1111/prd.12150

3. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P (1991) The soft tissue barrier at implants and teeth. Clin Oral Implants Res 2:81–90. https://doi.org/10.1034/j.1600-0501.1991.020206.x

4. Mombelli A, Decaillet F (2011) The characteristics of biofilms in peri-implant disease. J Clin Periodontol 38(Suppl 11):203–213. https://doi.org/10.1111/j.1600-051X.2010.01666.x

5. Laosuwan K, Epasinghe DJ, Wu Z et al (2018) Comparison of biofilm formation and migration of Streptococcus mutans on tooth roots and titanium miniscrews. Clin Exp Dent Res 4:40–47. https://doi.org/10.1002/cre2.105

6. Camposilvan E, Marro FG, Mestra A, Anglada M (2015) Enhanced reliability of yttria-stabilized zirconia for dental applications. Acta Biomater 17:36–46. https://doi.org/10.1016/j.actbio.2015.01.023

7. Tang K, Luo ML, Zhou W et al (2023) The integration of peri-implant soft tissues around zirconia abutments: challenges and strategies. Bioact Mater 27:348–361. https://doi.org/10.1016/j.bioactmat.2023.04.008

8. Hui WL, Perrotti V, Iaculli F et al (2020) The emerging role of cold atmospheric plasma in implantology: a review of the literature. Nanomaterials (Basel) 10:1505. https://doi.org/10.3390/nano10081505

9. Wagner G, Eggers B, Duddeck D et al (2022) Influence of cold atmospheric plasma on dental implant materials—an in vitro analysis. Clin Oral Investig 26:2949–2963. https://doi.org/10.1007/s00784-021-04277-w

10. Zheng M, Yang Y, Liu XQ et al (2015) Enhanced biological behavior of in vitro human gingival fibroblasts on cold plasma-treated zirconia. PLoS One 10:e0140278. https://doi.org/10.1371/journal.pone.0140278

11. Shahmiri R, Standard OC, Hart JN, Sorrell CC (2018) Optical properties of zirconia ceramics for esthetic dental restorations: a systematic review. J Prosthet Dent 119:36–46. https://doi.org/10.1016/j.prosdent.2017.04.018

12. Figueira LW, Panariello BHD, Koga-Ito CY et al (2021) Low-temperature plasma as an approach for inhibiting a multi-species cariogenic biofilm. Appl Sci 11:570. https://doi.org/10.3390/app11020570

13. Park C, Park SW, Yun KD et al (2018) Effect of plasma treatment and its post process duration on shear bonding strength and antibacterial effect of dental zirconia. Materials (Basel) 11:2233. https://doi.org/10.3390/ma11112233

14. Henningsen A, Smeets R, Heuberger R et al (2018) Changes in surface characteristics of titanium and zirconia after surface treatment with ultraviolet light or non-thermal plasma. Eur J Oral Sci 126:126–134. https://doi.org/10.1111/eos.12400

15. Yang Y, Zheng M, Yang Y et al (2020) Inhibition of bacterial growth on zirconia abutment with a helium cold atmospheric plasma jet treatment. Clin Oral Investig 24:1465–1477. https://doi.org/10.1007/s00784-019-03179-2

16. Yang Y, Zheng M, Jia YN et al (2021) Time-dependent reactive oxygen species inhibit Streptococcus mutans growth on zirconia after a helium cold atmospheric plasma treatment. Mater Sci Eng C Mater Biol Appl 120:111633. https://doi.org/10.1016/j.msec.2020.111633

17. Nothdurft FP, Fontana D, Ruppenthal S et al (2015) Differential behavior of fibroblasts and epithelial cells on structured implant abutment materials: a comparison of materials and surface topographies. Clin Implant Dent Relat Res 17:1237–1249. https://doi.org/10.1111/cid.12253

18. Teughels W, Van Assche N, Sliepen I, Quirynen M (2012) Influence of topography and hydrophilicity on initial oral biofilm formation on microstructured titanium surfaces in vitro. Clin Oral Implants Res 23:301–307. https://doi.org/10.1111/j.1600-0501.2011.02184.x

19. Jha N, Ryu JJ, Choi EH, Kaushik NK (2017) Generation and role of reactive oxygen and nitrogen species induced by plasma, lasers, chemical agents, and other systems in dentistry. Oxid Med Cell Longev 2017:7542540. https://doi.org/10.1155/2017/7542540

20. Muguerza-Guevara K, Cortés-Acha B, García-García M et al (2025) In vivo analysis of early biofilm development and cell viability on implant-mimicking abutments at 24 h, 48 h, and 7 days. BMC Oral Health 25:1201. https://doi.org/10.1186/s12903-025-06580-4