

Research Article
Implant Science



Safety evaluation of atmospheric pressure plasma jets in *in vitro* and *in vivo* experiments

Ji-Yoon Lee ^{1,2,†}, Shin-Young Park ^{3,†}, Kyoung-Hwa Kim ¹, Sung-Young Yoon ⁴,
Gon-Ho Kim ⁵, Yong-Moo Lee ¹, Yang-Jo Seol ^{1*}

¹Department of Periodontology and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea

²Department of Dentistry, Catholic Kwandong University, International St. Mary's hospital, Incheon, Korea

³Department of Dental Science and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Korea

⁴Plasma Technology Research Center, National Fusion Research Institute, Gunsan, Korea

⁵Department of Energy Systems (Nuclear) Engineering, Seoul National University School of Engineering, Seoul, Korea

OPEN ACCESS

Received: Nov 20, 2020

Revised: Jan 20, 2021

Accepted: Mar 5, 2021

***Correspondence:**

Yang-Jo Seol

Department of Periodontology and Dental Research Institute, School of Dentistry, Seoul National University, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea.

E-mail: yjseol@snu.ac.kr

Tel: +82-2-2072-0308

Fax: +82-2-744-0051

[†]Shin-Young Park and Ji-Yoon Lee contributed equally to this study.

Copyright © 2021. Korean Academy of Periodontology

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>).

ORCID iDs

Ji-Yoon Lee

<https://orcid.org/0000-0001-5246-9191>

Shin-Young Park

<https://orcid.org/0000-0002-3776-4130>

Kyoung-Hwa Kim

<https://orcid.org/0000-0001-5611-1242>

Sung-Young Yoon

<https://orcid.org/0000-0003-0118-8474>

Gon-Ho Kim

<https://orcid.org/0000-0002-2546-8350>

Yong-Moo Lee

<https://orcid.org/0000-0002-5619-3847>

Yang-Jo Seol

<https://orcid.org/0000-0002-2076-5452>

ABSTRACT

Purpose: The atmospheric pressure plasma jet (APPJ) has been introduced as an effective disinfection method for titanium surfaces due to their massive radical generation at low temperatures. Helium (He) has been widely applied as a discharge gas in APPJ due to its bactericidal effects and was proven to be effective in our previous study. This study aimed to evaluate the safety and effects of He-APPJ application at both the cell and tissue levels.

Methods: Cellular-level responses were examined using human gingival fibroblasts and osteoblasts (MC3T3-E1 cells). He-APPJ was administered to the cells in the experimental group, while the control group received only He-gas treatment. Immediate cell responses and recovery after He-APPJ treatment were examined in both cell groups. The effect of He-APPJ on osteogenic differentiation was evaluated via an alkaline phosphatase activity assay. *In vivo*, He-APPJ treatment was administered to rat calvarial bone and the adjacent periosteum, and samples were harvested for histological examination.

Results: He-APPJ treatment for 5 minutes induced irreversible effects in both human gingival fibroblasts and osteoblasts *in vitro*. Immediate cell detachment of human gingival fibroblasts and osteoblasts was shown regardless of treatment time. However, the detached areas in the groups treated for 1 or 3 minutes were completely repopulated within 7 days. Alkaline phosphatase activity was not influenced by 1 or 3 minutes of plasma treatment, but was significantly lower in the 5 minute-treated group ($P=0.002$). *In vivo*, He-APPJ treatment was administered to rat calvaria and periosteum for 1 or 3 minutes. No pathogenic changes occurred at 7 days after He-APPJ treatment in the He-APPJ-treated group compared to the control group (He gas only).

Conclusions: Direct He-APPJ treatment for up to 3 minutes showed no harmful effects at either the cell or tissue level.

Keywords: Animal experimentation; Bone and bones; Fibroblasts; Osteoblasts; Periosteum; Plasma gases

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2020R1A2C1007536). This work was supported by the Research Resettlement Fund for new faculty, the Creative-Pioneering Researchers Program of Seoul National University.

Author Contributions

Conceptualization: Gon-Ho Kim, Yang-Jo Seol, Kyoung-Hwa Kim; Formal analysis: Ji-Yoon Lee; Investigation: Ji-Yoon Lee, Kyoung-Hwa Kim; Methodology: Sung-Young Yoon, Kyoung-Hwa Kim, Yang-Jo Seol; Project administration: Gon-Ho Kim, Yang-Jo Seol; Writing - original draft: Ji-Yoon Lee, Shin-Young Park, Yang-Jo Seol; Writing - review & editing: Yong-Moo Lee.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

INTRODUCTION

Pathogenic bacteria, which exist as biofilms on dental implant surfaces, are the main cause of peri-implant diseases, inducing inflammatory responses that play a critical role in bone loss around implants and implant failure in severe cases [1-3]. In this context, disinfection of pathogenic bacteria is key to the treatment of peri-implant disease.

The atmospheric pressure plasma jet (APPJ) has drawn attention in the biomedical field due to the sterilizing effects of its plasma [4]. A plasma jet consists of a gas nozzle with electrodes. The plasma is ionized gas generated inside the nozzle and transported to the object to be treated by a gas flow. The features of plasma depend on the electrode configurations, type of gas, and frequency of the applied voltage. APPJ is a non-thermal plasma known to be highly effective for bactericidal irradiation and sterilization, wound healing, blood coagulation, material surface modifications, and therapeutic applications including in cancer [5,6]. The charged particles and reactive oxygen and nitrogen species generated by plasma play a pivotal role in biological responses [7-9]. The advantage of plasma with a low temperature is its selective effects on living system [10]. Guerrero-Preston et al. [11] applied a helium gas-based APPJ (He-APPJ) to head and neck squamous cell carcinoma cells and confirmed selective impairment of cancer cells with a minimal effect on normal oral cavity cells.

In our previous study, we suggested using He-APPJ to disinfect peri-implantitis lesions [12]. He-APPJ treatment on a sandblasted and acid-etched (SLA) surface resulted in removal of bacteria from contaminated titanium surfaces without damaging the titanium surface. Interestingly, the bactericidal effect of He-APPJ was not limited to the central area, where the beam of the plasma jet was targeted, but was also observed in the marginal area. However, the effect of plasma treatment on the normal oral cavity and periodontium has not yet established at this bactericidal dose of He-APPJ.

This study aimed to evaluate the safety of He-APPJ in *in vitro* and *in vivo* conditions at the effective dose for the removal of bacteria from SLA titanium surfaces. In order to mimic the oral cavity and periodontal complex, safety tests were performed on gingival fibroblasts and osteoblasts at the cellular level and periosteal and bone responses at the tissue level. For the control group, He-gas blowing only, without plasma jet generation, was applied.

MATERIALS AND METHODS

He-APPJ device

The design of the APPJ apparatus (Figure 1) was described by Yoon et al. [13]. The APPJ source consists of a very low frequency (VLF)-driven 0.2-mm tungsten electrode surrounded by an alumina (Al₂O₃) tube with an outer diameter of 0.6 mm and a coaxial quartz gas guiding tube with an inner diameter of 1 mm and a thickness of 1 mm. He was used as the discharge gas. The discharge gas was injected into the gap between the alumina and quartz tubes and then evacuated into the open air. The gas flow rate was maintained at 5 standard liters per minute and controlled by a mass flow controller (TN 2900; Brooks Instrument, Hatfield, PA, USA). The VLF was generated by using a function generator connected to a voltage amplifier and set to 7 kV under a pulse of 10 kHz (MF plasma power supply; Dawonsys, Ansan, Korea). The plasma device was fixed on a stand, which prevented the device from moving and maintained the distance between the plasma tip and specimens. The distance from the plasma tip to the specimens was 30 mm.

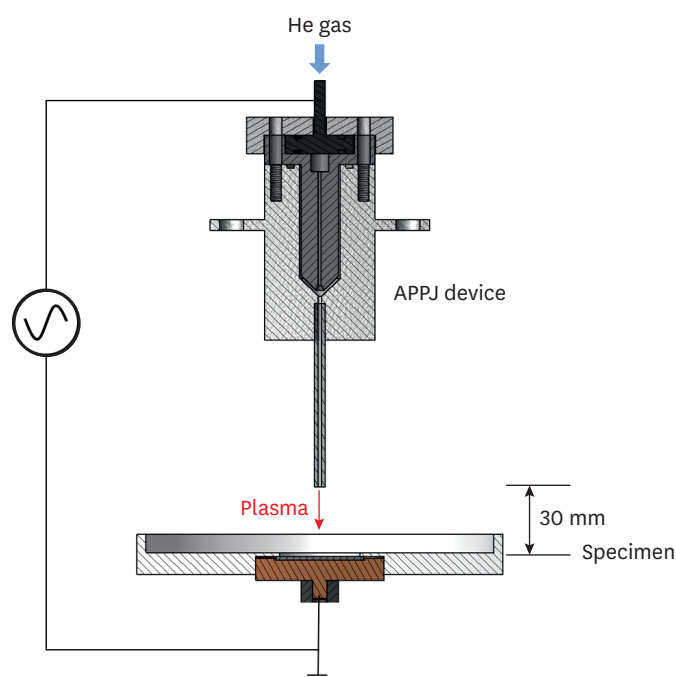


Figure 1. He-APPJ device (a very low frequency-driven 0.2-mm tungsten electrode surrounded by an alumina tube with an outer diameter of 0.6 mm and a coaxial quartz gas guiding tube with an inner diameter of 1 mm and a thickness of 1 mm, and a voltage of 7 kV under a pulse of 10 kHz). He: helium, APPJ: atmospheric pressure plasma jet.

In vitro experiments

Cell preparations

Human gingival fibroblasts (ScienCell Research Laboratories Inc., Carlsbad, CA, USA) and the mouse osteoblast-like cell line MC3T3-E1 were used in this study. Cells were cultured in alpha minimum essential medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution (Gibco™, Dublin, Ireland). Cells were incubated at 37°C in humidified air with 5% CO₂. The medium was changed twice a week. Cells were used at passages 2 to 3.

He-APPJ application to cells

To investigate the cellular response to He-APPJ treatment, He-APPJ was used to irradiate gingival fibroblasts and osteoblasts. A total of 1×10^4 cells were seeded in 35-mm culture dishes and cultured to confluence. Cells were treated with He-APPJ for 0, 1, 3, or 5 minutes. After He-APPJ treatment, cells were stained with multiple staining solutions and observed with light microscopy on days 0 and 7.

Alkaline phosphatase activity

The alkaline phosphatase (ALP) activity of cells was examined after He-APPJ treatment. MC3T3-E1 osteoblasts (1×10^4) were seeded in plates. After the cells became confluent, they were treated with plasma for 0, 1, 3, or 5 minutes. Then, the cells were incubated at 37°C in 5% CO₂ for 7 days, and ALP activity was measured in accordance with the manufacturer's instructions (Anaspec Co., Fremont, CA, USA). The cell lysates and ALP substrates were transferred to plates and incubated at 37°C for 30 minutes. The reaction was terminated by adding a stop solution. The p-nitrophenol (pNP) product formed by enzymatic hydrolysis of p-nitrophenylphosphate was measured at 405 nm using an absorbance microplate reader.

The protein concentrations in the samples were measured using a protein assay kit (iNtRON Biotechnology, Seongnam, Korea). ALP activity was expressed as the concentration of pNP transformed per microgram of protein [14].

In vivo experiments

Animals

A total of 21 male Sprague-Dawley rats (8 weeks old, weighing 240–320 g) were used in this study. Animals were housed in specific pathogen-free facilities. Animals were cared for and treated in accordance with guidelines established by the Seoul National University Institutional Animal Care and Use Committee. The animal research protocol was approved by the Institute of Laboratory Animal Resources, Seoul National University (SNU-150626-2).

Surgical procedures

Surgical procedures were performed under general anesthesia with an intraperitoneal injection of a mixture of tiletamine/zolazepam (Zoletil, Virbac S.A., Carros, France; 8 mg/kg) and xylazine (Rompun, Bayer AG, Leverkusen, Germany; 2.25 mg/kg). The heads of the experimental rats were shaved and sterilized with iodine, and infiltration anesthesia was performed. A horizontal incision was made from left to right between the ears. A midline incision was made starting between the eyes and extending dorsally to meet the horizontal ear-to-ear incision. The flap was lifted and fixed with 4-0 silk sutures. Two symmetrical 5-mm calvarial circles were made on each side of the midline with a trephine bur (3i Implant Innovation, Palm Beach, FL, USA) under copious saline irrigation. He-APPJ treatment was applied at the center of the calvarial circles and at the corner of the exposed periosteum (Figure 2) depending on the experimental group. The experimental groups were as follows: 1) He-APPJ treatment for 1 minute, 2) He-APPJ treatment for 3 minutes, 3) He treatment only for 1 minute, and 4) He treatment only for 3 minutes. After He-APPJ treatment, the periosteum was sutured first with 5-0 chromic gut, and the exposed skin was sutured with 4-0 silk. Intramuscular injection of 30 mg/kg cefazolin (Pfizer, New York, NY, USA) was given to the animals for 2 days after surgery. The animals were sacrificed after 7 and 14 days for histological examinations.

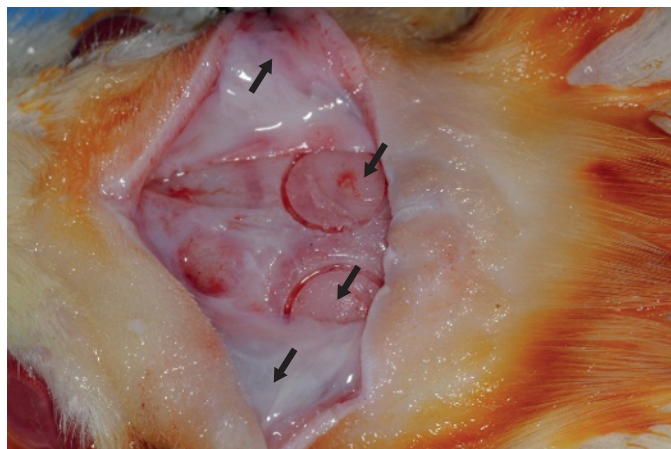


Figure 2. Rat calvaria and adjacent periosteum were prepared for plasma treatment under general anesthesia. Two identical 5-mm-diameter circular defects were created symmetrically, and He-APPJ or He gas only was administered at the center of each circle and the corners of the periosteal flap. The plasma-treated areas are indicated by black arrows.

He: helium, APPJ: atmospheric pressure plasma jet.

Histological analysis

The specimens were decalcified with 10% EDTA solution for 2 weeks prior to dehydration in ascending ethanol concentrations (50%, 70%, 96%, and 100%) and embedding in paraffin. A 5- μ m-thick coronal section through the center of the circular defect was obtained and stained with hematoxylin and eosin. The prepared specimens were examined by light microscopy.

Statistical analysis

The data are presented as means \pm standard deviations. Statistical comparisons were performed using 1-way analysis of variance with the Bonferroni *post hoc* test. *P* values of < 0.05 were considered to indicate statistical significance.

RESULTS

The effect of He-APPJ on gingival fibroblasts and osteoblasts

He-APPJ treatment of human gingival fibroblasts resulted in a circular, ring-shaped cell detachment area at the edge of the area directly exposed to plasma, while the cells within the edge were intact (Figure 3). The total cell detachment area increased with treatment time. During recovery, the detachment area was completely repopulated by fibroblasts in all groups except for the 5-minute treatment group, in which cells were located only inside the circle and were not attached outside the circle (black circle: direct plasma treatment area).

Osteoblasts showed a similar response at the time of He-APPJ treatment—that is, a circular, ring-shaped cell detachment area was observed (Figure 4). Unlike fibroblast proliferation, osteoblast proliferation recovered fully within 7 days, including in the 5-minute treatment group. However, the recovered osteoblasts in the 5-minute treatment group showed a significant reduction in ALP activity compared to those in the 0-, 1-, and 3-minute treatment groups (Figure 5; $P=0.002$).

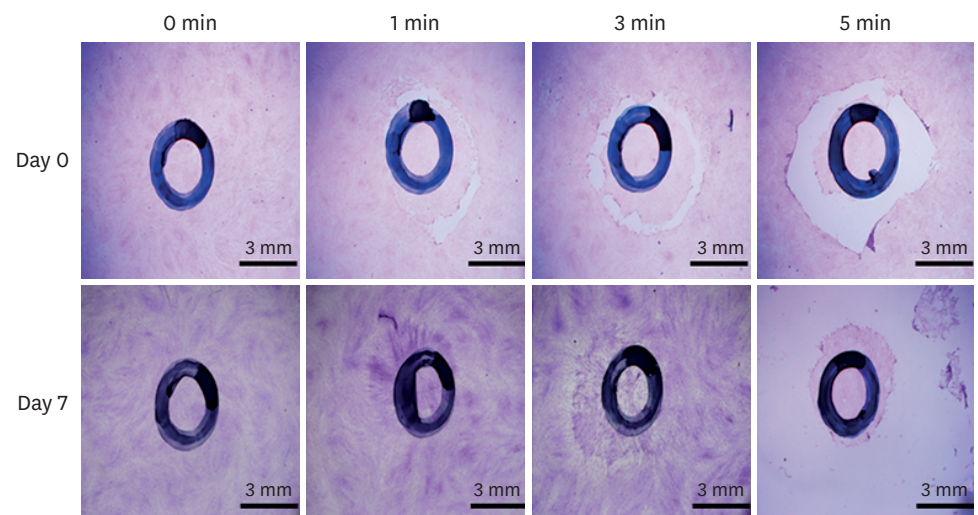


Figure 3. Histological evaluation of gingival fibroblasts after plasma application on day 0 (upper) and day 7 (lower). On day 0, circular and ring-shaped cell detachment areas occurred at the periphery of the exposed area. Cell detachment increased as treatment time increased. On day 7, the detached area was completely repopulated with fibroblasts in all groups except for the 5-minute treatment group, in which cells were located only inside the circle and were not attached outside the circle (black circle: direct plasma treatment area) (original magnification, $\times 2$).

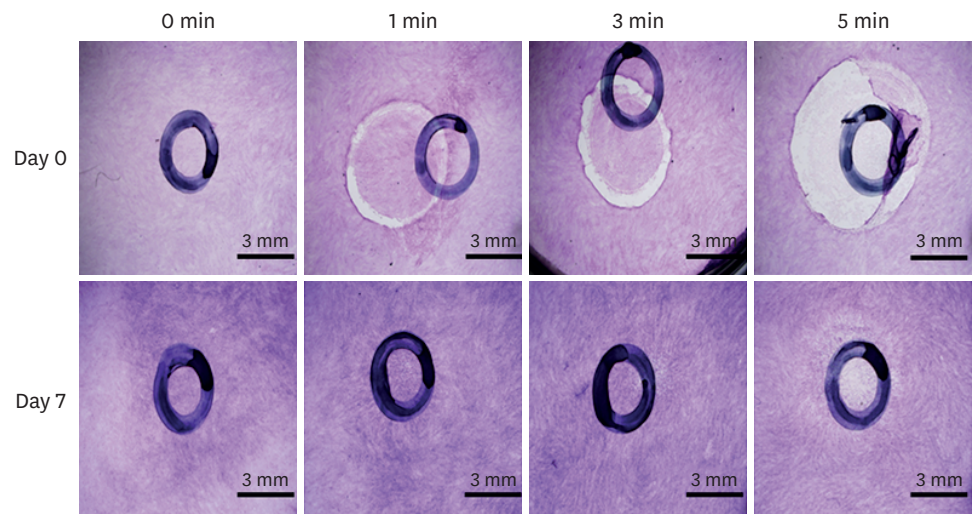


Figure 4. Histological evaluation of osteoblasts and MC3T3-E1 cells after plasma application on days 0 (upper) and 7 (lower). On day 0, a circular, ring-shaped cell detachment area was found, and the cell detachment area increased with increasing plasma treatment time. On day 7, all detached areas were completely repopulated with osteoblasts (black circle: direct plasma treatment area) (original magnification, $\times 2$).

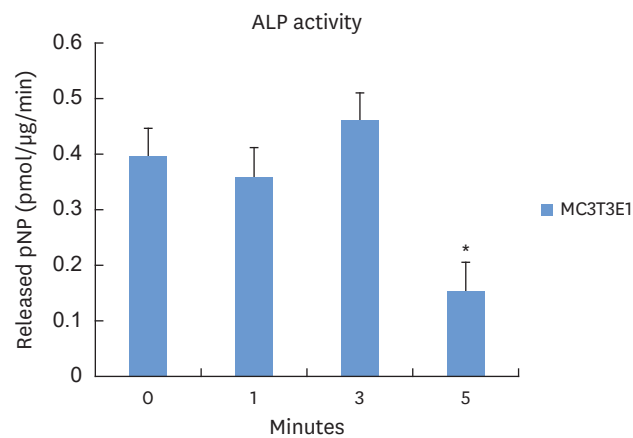


Figure 5. ALP activity of osteoblasts 7 days after plasma treatment for 0, 1, 3, and 5 minutes. ALP activity was not significantly different between the 0-, 1-, and 3-minute treatment groups. However, the 5-minute treatment group showed a significant decrease in activity compared with the other groups ($P=0.002$).

Data are reported as the means \pm standard deviations. Experiments were performed in triplicate.

ALP: alkaline phosphatase, pNP: p-nitrophenol.

* $P < 0.05$ versus control (0 minutes), 1-way analysis of variance with the Bonferroni *post hoc* test.

The effect of He-APPJ on healthy bone and periosteum

Based on the *in vitro* experiment, the safe He-APPJ treatment time *in vivo* was determined to be at most 3 minutes. *In vivo* animal experiments demonstrated no visible damage or changes after He-APPJ treatment in any treatment group, as shown in the photographs in Figure 2.

All animals healed uneventfully after He-APPJ treatment. None of the animals died after He-APPJ treatment or He-gas treatment. No wound dehiscence or inflammatory soft tissue changes were observed during the healing period.

Histologically, rat calvarial bone showed no delayed or impaired bone healing in the He-APPJ group compared with the He gas-only group regardless of treatment time (Figures 6 and 7). The defect border (blue arrow) was clear at day 7 and had partially healed at day 7. The cortical

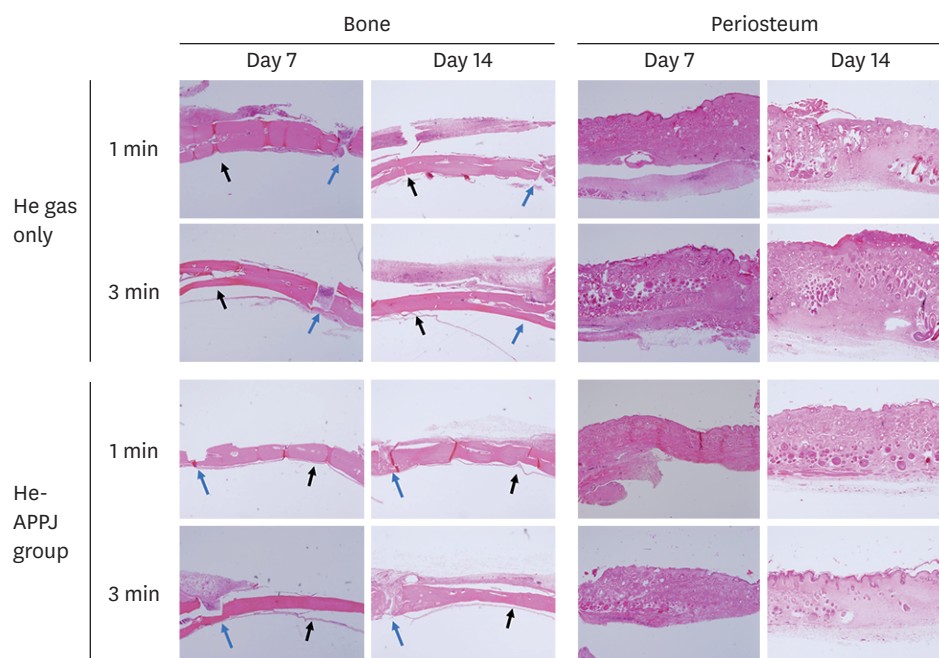


Figure 6. Histological evaluation of rat calvaria and periosteum at 7 and 14 days after He-APPJ or He gas treatment for 1 minute or 3 minutes. In the calvarial bone, no visible damage or change was detected in the He-APPJ-treated group compared to the He gas-only group on days 7 and 14. The defect border was prominent on day 7 but had healed on day 14. In the periosteum, no ulcerative healing or inflammatory cell infiltrate was observed on days 7 and 14 (black arrows, estimated helium-treated area; blue arrows, defect border) (original magnification, $\times 4$).

He: helium, APPJ: atmospheric pressure plasma jet.

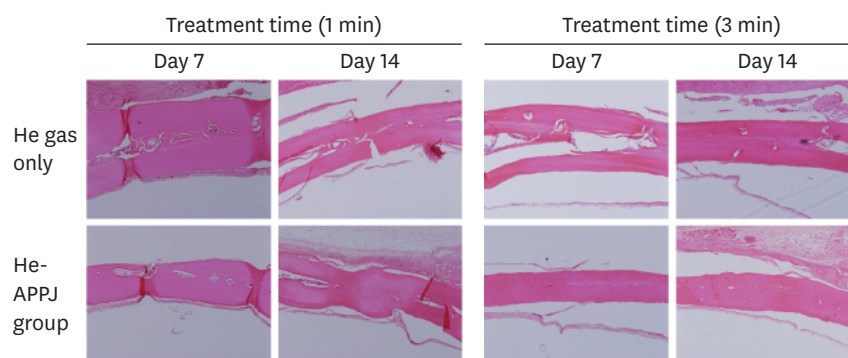


Figure 7. High magnified images of helium-treated areas (original magnification, $\times 10$).

He: helium, APPJ: atmospheric pressure plasma jet.

bone and marrow space was intact in the He or He-APPJ treated site (black arrow) at day 7 and 14. In the periosteum, He-APPJ treatment induced no epidermal or dermal changes, and the continuity of the basement membrane was well maintained in all treatment groups.

DISCUSSION

In this study, we examined the effect of He-APPJ at the cellular and tissue levels. In order to simulate the oral cavity, which consists of gum, mucosa, and bone tissue, human gingival fibroblasts and osteoblasts were used for *in vitro* experiments. He-APPJ treatment for 5

minutes induced irreversible changes in gingival fibroblasts not osteoblasts. However, even though the osteoblasts regrew, the osteogenic features of osteoblasts were significantly reduced after 5 minutes of He-APPJ treatment. *In vivo*, He-APPJ treatment for under 3 minutes showed no harmful effects in histological examinations.

Cellular reactions to He-APPJ treatment have been reported to range from cell detachment to apoptosis, depending on the irradiation dose and cell type [15-17]. He-APPJ treatment of human periodontal ligament cells did not induce cell death, although cellular responses such as redox status were influenced by the microenvironment. [18]. In vascular cells, cell detachment was observed at a lower dose, apoptosis at a higher dose, and necrosis at a very high dose [19]. The viability of osteoblasts was unaffected by direct He-APPJ treatment, while the number of mesenchymal stromal cells was decreased by plasma treatment but recovered within 72 hours [20].

Previous studies reported that plasma treatment of wound surfaces caused no visible or microscopic damage to accelerate wound healing [21,22]. Bender and colleagues tested plasma treatment by the hen's egg test to determine the irritation potential of plasma and found partially or completely reversible changes depending on the intensity of the irritation [21]. In an animal model, Ermolaeva et al. [23] showed that APPJ treatment significantly reduced the pathogenic bacterial load on the wound surface in a superficial slash wound defect and increased the wound closure rate. However, Nastuta et al. [24] found that He-APPJ treatment accelerated skin re-epithelization, whereas the superficial dermis recovered slowly. Therefore, the response to He-APPJ treatment at the bone tissue level is unclear.

Shashurin et al. [25] suggested a classification scheme for plasma jet treatment according to the cellular response (intense, medium, or mild): intense treatment results in direct contact between the plasma jet and cells by displacing the culture medium from the point of exposure to the plasma jet, leading to cell death; medium treatment induces detachment of cells from the extracellular matrix, because the cell culture medium forms an interface between the cells and the plasma jet; and mild treatment causes a modest cellular response with no detachment. According to this classification, He-APPJ produced medium-level cellular changes when the He-APPJ treatment time was 3 minutes or less. Similarly, previous studies also showed immediate cell detachment after plasma treatment and successful reattachment after certain healing periods [26]. Regarding the mechanism of cell detachment, Xu and colleagues [27] recently showed a correlation between the presence of hydroxyl radicals in the gas phase of the plasma and the cell detachment area.

Unlike osteoblasts, the proliferation of gingival fibroblasts treated with He-APPJ for 5 minutes was not recovered. Some studies have referred to this continuous cell detachment after plasma radiation exposure as "delayed cell detachment" [28,29]. The continuous loss of cells is not due to reversible cell detachment, but to the apoptotic behavior of the cells after plasma treatment. The mechanism of delayed cell detachment involves the reaction of intracellular reactive oxygen species with cell adhesion molecules [28,30]. Others have suggested that delayed cell detachment is related to the desiccation of cells from the blowing gas during APPJ treatment [19,25]. According to Kieft et al. [26], the mammalian cell reactions to plasma are cell detachment, apoptosis, and cell necrosis. In several studies, cells were not detached, but necrotized under high-power plasma treatment [26,30]. That is, the response of gingival fibroblasts in the 5-minute treatment group was related to the exposure time, the distance from the cells to the plasma tip, and the thickness of the medium covering the cells, not to the He-APPJ generation setting [5].

In the current study, osteoblasts completely recovered in the 5-minute treatment group, which was similar to the results reported by Canal et al. [20]. That group applied these settings for bone cancer therapy and demonstrated plasma-induced selective bone cell death [20]. Interestingly, both osteoblasts and osteosarcoma cells exhibited slowed proliferation at the time of He-APPJ treatment, but osteosarcoma cells did not recover after 72 hours of culture, while the viability of osteoblasts was unaffected. However, the osteogenic activity of osteoblasts was significantly affected by He-APPJ treatment in the 5-minute treatment group. He-APPJ treatment for more than 5 minutes might cause permanent damage to cells and fail to enhance osteogenic activity. One of the possible mechanisms of this damage is desiccation of cells during plasma treatment, as mentioned above. Desiccation of specimens was unavoidable during plasma treatment, especially when plasma exposure lasted for more than 5 minutes.

In our previous study, 3 minutes of He-APPJ treatment was effective for the decontamination of *Porphyromonas gingivalis* biofilm-covered SLA titanium discs [12]. Bacterial counts significantly decreased after 3 minutes of treatment and were under the detectable range after 5 minutes. SLA surfaces showed no changes after He-APPJ treatment regardless of treatment time. No viable bacterial cells were observed in the area directly exposed to He-APPJ treatment after 3 minutes or at either the directly or indirectly exposed areas after 5 minutes. Taken together, these results indicate that 5 minutes of He-APPJ treatment induced intense bacterial and cellular responses, such as cell death, permanent cell damage, and bacterial rupture. Therefore, in this study, 3 minutes of He-APPJ treatment was considered appropriate for *in vivo* applications. He-APPJ treatment of rat calvarial bone and adjacent periosteum for 3 minutes or less resulted in no microscopic damage or impaired healing *in vivo* after 7 and 14 days of healing. The results of other *in vivo* studies correspond to this result, indicating that no specimens showed visible damage after plasma exposure [31-34].

This study had some limitations related to the experimental design. This study only demonstrated the safety related to He-APPJ around normal tissues, and inflamed tissues around contaminated SLA titanium could show different responses to He-APPJ. Inflamed gingival tissues are vulnerable and easily penetrated by light forces. Cells stimulated by proinflammatory cytokines might not be able to tolerate 3 minutes of He-APPJ treatment. However, based on the results of the previous bacterial disinfection study, 3 minutes of He-APPJ should be applied to defects and titanium for desirable effects. Therefore, for clinical applications of He-APPJ on peri-implantitis, more delicate settings of He-APPJ are needed, and these settings should be tested in various conditions in further research.

In conclusion, He-APPJ treatment for 3 minutes is the proper dosage to obtain the antibacterial effects of He-APPJ treatment without any permanent damage to adjacent cells and tissues.

REFERENCES

1. Heitz-Mayfield LJ, Lang NP. Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. *Periodontol* 2000 2010;53:167-81.
[PUBMED](#) | [CROSSREF](#)
2. Zhu B, Meng H, Huang B, Chen Z, Lu R. Detection of *T. forsythia* and other important bacteria in crestal and subcrestal implants with ligature-induced peri-implant infection in dogs. *J Periodontol* 2019;90:306-13.
[PUBMED](#) | [CROSSREF](#)

3. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. *Periodontol 2000* 1998;17:63-76.
[PUBMED](#) | [CROSSREF](#)
4. Ehlbeck J, Brandenburg R, von Woedtke T, Krohmann U, Stieber M, Weltmann KD. PLASMOSE - antimicrobial effects of modular atmospheric plasma sources. *GMS Krankenhhyg Interdiszip* 2008;3:Doc14.
[PUBMED](#)
5. Yonson S, Coulombe S, Leveille V, Leask R. Cell treatment and surface functionalization using a miniature atmospheric pressure glow discharge plasma torch. *J Phys D Appl Phys* 2006;39:3508-13.
[CROSSREF](#)
6. Lupu AR, Georgescu N, Călugăru A, Cremer L, Szegli G, Kerek F. The effects of cold atmospheric plasma jets on B16 and COLO320 tumoral cells. *Roum Arch Microbiol Immunol* 2009;68:136-44.
[PUBMED](#)
7. Mendis DA, Rosenberg M, Azam F. A note on the possible electrostatic disruption of bacteria. *IEEE Trans Plasma Sci* 2000;28:1304-6.
[CROSSREF](#)
8. Laroussi M. Nonthermal decontamination of biological media by atmospheric-pressure plasmas: review, analysis, and prospects. *IEEE Trans Plasma Sci* 2002;30:1409-15.
[CROSSREF](#)
9. Montie TC, Roth R. An overview of research using the one atmosphere uniform glow discharge plasma for sterilization of surfaces and materials. *IEEE Trans Plasma Sci* 2000;28:41-50.
[CROSSREF](#)
10. Kim JH, Lee MA, Han GJ, Cho BH. Plasma in dentistry: a review of basic concepts and applications in dentistry. *Acta Odontol Scand* 2014;72:1-12.
[PUBMED](#) | [CROSSREF](#)
11. Guerrero-Preston R, Ogawa T, Uemura M, Shumulinsky G, Valle BL, Pirini F, et al. Cold atmospheric plasma treatment selectively targets head and neck squamous cell carcinoma cells. *Int J Mol Med* 2014;34:941-6.
[PUBMED](#) | [CROSSREF](#)
12. Lee JY, Kim KH, Park SY, Yoon SY, Kim GH, Lee YM, et al. The bactericidal effect of an atmospheric-pressure plasma jet on *Porphyromonas gingivalis* biofilms on sandblasted and acid-etched titanium discs. *J Periodontal Implant Sci* 2019;49:319-29.
[PUBMED](#) | [CROSSREF](#)
13. Yoon SY, Kim KH, Seol YJ, Kim SJ, Bae B, Huh SR, et al. Effects of metastable species in helium and argon atmospheric pressure plasma jets (APPJs) on inactivation of periodontopathogenic bacteria. *J Korean Phys Soc* 2016;68:1176-91.
[CROSSREF](#)
14. Park SY, Kim KH, Shin SY, Koo KT, Lee YM, Seol YJ. Dual delivery of rhPDGF-BB and bone marrow mesenchymal stromal cells expressing the *BMP2* gene enhance bone formation in a critical-sized defect model. *Tissue Eng Part A* 2013;19:2495-505.
[PUBMED](#) | [CROSSREF](#)
15. Kalghatgi S, Kelly CM, Cerchar E, Torabi B, Alekseev O, Fridman A, et al. Effects of non-thermal plasma on mammalian cells. *PLoS One* 2011;6:e16270.
[PUBMED](#) | [CROSSREF](#)
16. Stoffels E, Sakiyama Y, Graves DB. Cold atmospheric plasma: charged species and their interactions with cells and tissues. *IEEE Trans Plasma Sci* 2008;36:1441-57.
[CROSSREF](#)
17. Volotskova O, Shashurin A, Stepp MA, Pal-Ghosh S, Keidar M. Plasma-controlled cell migration: localization of cold plasma-cell interaction region. *Plasma Med* 2011;1:85-92.
[CROSSREF](#)
18. Virard F, Cousty S, Cambus JB, Valentin A, Kémoun P, Clément F. Cold atmospheric plasma induces a predominantly necrotic cell death via the microenvironment. *PLoS One* 2015;10:e0133120.
[PUBMED](#) | [CROSSREF](#)
19. Stoffels E, Roks AJM, Deelman LE. Delayed effects of cold atmospheric plasma on vascular cells. *Plasma Process Polym* 2008;5:599-605.
[CROSSREF](#)
20. Canal C, Fontelo R, Hamouda I, Guillem-Marti J, Cvelbar U, Ginebra MP. Plasma-induced selectivity in bone cancer cells death. *Free Radic Biol Med* 2017;110:72-80.
[PUBMED](#) | [CROSSREF](#)
21. Bender C, Matthes R, Kindel E, Kramer A, Lademann J, Weltmann KD, et al. The irritation potential of nonthermal atmospheric pressure plasma in the HET-CAM. *Plasma Process Polym* 2010;7:318-26.
[CROSSREF](#)

22. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. *Plasma Process Polym* 2008;5:503-33.
CROSSREF
23. Ermolaeva SA, Varfolomeev AF, Chernukha MY, Yurov DS, Vasiliev MM, Kaminskaya AA, et al. Bactericidal effects of non-thermal argon plasma in vitro, in biofilms and in the animal model of infected wounds. *J Med Microbiol* 2011;60:75-83.
PUBMED | CROSSREF
24. Nastuta AV, Topala I, Grigoras C, Pohoata V, Popa G. Stimulation of wound healing by helium atmospheric pressure plasma treatment. *J Phys D Appl Phys* 2011;44:105204.
CROSSREF
25. Shashurin A, Keidar M, Bronnikov S, Jurjus RA, Stepp MA. Living tissue under treatment of cold plasma atmospheric jet. *Appl Phys Lett* 2008;93:181501.
CROSSREF
26. Kieft IE, Broers JL, Caubet-Hilloutou V, Slaaf DW, Ramaekers FC, Stoffels E. Electric discharge plasmas influence attachment of cultured CHO K1 cells. *Bioelectromagnetics* 2004;25:362-8.
PUBMED | CROSSREF
27. Xu D, Luo X, Xu Y, Cui Q, Yang Y, Liu D, et al. The effects of cold atmospheric plasma on cell adhesion, differentiation, migration, apoptosis and drug sensitivity of multiple myeloma. *Biochem Biophys Res Commun* 2016;473:1125-32.
PUBMED | CROSSREF
28. Fridman G, Shereshevsky A, Jost MM, Brooks AD, Fridman A, Gutsol A, et al. Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. *Plasma Chem Plasma Process* 2007;27:163-76.
CROSSREF
29. Lee JH, Kim KN. Effects of a nonthermal atmospheric pressure plasma jet on human gingival fibroblasts for biomedical application. *BioMed Res Int* 2016;2016:2876916.
PUBMED | CROSSREF
30. Kieft IE, Darios D, Roks AJM, Stoffels E. Plasma treatment of mammalian vascular cells: a quantitative description. *IEEE Trans Plasma Sci* 2005;33:771-5.
CROSSREF
31. Fridman G, Shereshevsky A, Peddinghaus M, Gutsol A, Vasilets V, Brooks A, et al. Bio-medical applications of non-thermal atmospheric pressure plasma. In *Proceedings of the 37th AIAA Plasmadynamics and Lasers Conference*; 2006 Jun 5-8; San Francisco, CA. Reston, VA: American Institute of Aeronautics and Astronautics; 2006.
CROSSREF
32. Fridman G, Peddinghaus M, Balasubramanian M, Ayan H, Fridman A, Gutsol A, et al. Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air. *Plasma Chem Plasma Process* 2006;26:425-42.
CROSSREF
33. Wu AS, Kalghatgi S, Dobrynin D, Sensenig R, Cerchar E, Podolsky E, et al. Porcine intact and wounded skin responses to atmospheric nonthermal plasma. *J Surg Res* 2013;179:e1-12.
PUBMED | CROSSREF
34. Liu D, Xiong Z, Du T, Zhou X, Cao Y, Lu X. Bacterial-killing effect of atmospheric pressure non-equilibrium plasma jet and oral mucosa response. *J Huazhong Univ Sci Technolog Med Sci* 2011;31:852-6.
PUBMED | CROSSREF