



Comparison study of root canal disinfection by cold plasma jet and photodynamic therapy

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ARTICLE INFO

Keywords:

Plasma
Photodynamic therapy
Root canal
Disinfection
Antimicrobial Photodynamic Therapy

ABSTRACT

Introduction: This study evaluated and compared the antibacterial effect of plasma and photodynamic therapy (PDT) in root canals infected with *Enterococcus faecalis*.

Methods: One hundred single-rooted extracted human teeth (no treatment) were selected and sterilized. Root canals of teeth were prepared with the crown-down technique and contaminated with *Enterococcus faecalis* after sterilization, and incubated for one week. To investigate the effect of disinfection, 60 specimens were randomly selected for three disinfection groups: He and He/O₂ plasma (n = 20) in 5-s groups (control, 2, 4, 6 and 8 min treatments) and PDT (n = 20) in 2-s groups (control and PDT treatment). Next, for comparison, 40 remaining teeth were divided into four groups: control, 8 min He plasma, 8 min He/O₂, and PDT. Results of antibacterial action were reported by CFU/mL analysis and the structural changes of biofilm were evaluated by SEM.

Results: All groups showed a significant reduction of bacteria after treatment, and He/O₂ plasma was more effective against *E. faecalis*, followed by PDT and He plasma respectively, further confirmed by SEM.

Conclusion: He/O₂ plasma is a strong sterilization method against *E. faecalis* biofilm and can, therefore, be an effective therapy in endodontics.

1. Introduction

Microbial infection has been recognized as a major etiologic factor in pulpal and periapical lesions. The effective control of microbial infection in the root canal system is the main purpose of endodontic treatment [1–3]. Traditional treatments such as mechanical debridement, chemical irrigation, ultrasound and different types of laser radiations can significantly reduce the population of bacteria inside the infected root canal. However, elimination of all bacteria from the root canal is hard. This is attributed to the complex morphology of root canal systems and the innate resistance properties of biofilms [4–9].

Multiple studies have shown that *Enterococcus faecalis* are commonly isolated bacteria associated with persistent periapical lesions. *E. faecalis* is a gram-positive facultative bacterium that can live in dental tubules for long periods of time under nutritional deprivation and often is found in the form of biofilm. The biofilm form of bacteria shows more resistance to antibacterial and antibodies than planktonic bacteria. When *E. faecalis* grows in the root canal system, it becomes more

resistant, although it can be easily destroyed in an open environment [10–16].

To eradicate *E. faecalis* biofilms from persistent root canal infections, more effective disinfection methods are needed. Researchers have recently introduced new methods that facilitate clinical work and result in efficient treatment.

Plasma is a new method for disinfection. It consists of charged particle, electron, photon, UV and free radicals. The components of plasma such as, single oxygen and free radicals have antibacterial properties. These species are able to inactivate cells and cause cell lysis by oxidation which result in decontamination and sterilization [17–19]. Plasma sterilization has widely been used in dentistry for tooth bleaching [20], removal of plaque [21], sterilization of dental equipment, implants [22,23] and disinfection of root canal [24].

Investigations on the effect of plasma in the root canal have shown heterogeneous results because of different parameters such as exposure time, feeding gas and plasma sources. Plasma was efficient against young and mature *E. faecalis* biofilms. Li et al reported that Ar/O₂

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<https://doi.org/10.1016/j.pdpdt.2019.04.023>

Received 2 January 2019; Received in revised form 2 March 2019; Accepted 22 April 2019

Available online 23 April 2019

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plasma more effectively killed *E. faecalis* compared to intracanal medicaments consisting Ca (OH)₂, 2% CHX, and Ca (OH)₂ plus 2% CHX (CH/CHX) in 3-week-old biofilm whereas, Ureyen Kaya et al showed plasma and NaOCl had similar results in 4-week-old biofilms [24–26]. In comparison the antibacterial effect of two different plasmas, Chen et al. reported that He/O₂ plasma was more effective in inactivating *Enterococcus faecalis* respect to He plasma [27].

Photodynamic therapy is the other method for disinfection that has been successful in endodontic disinfection. In this method, a light-sensitive compound (photosensitizer) is activated by the light with a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitizer to available oxygen, leading to the production of toxic oxygen species, such as atomic oxygen and free radicals, can kill a wide range of microbes [28–33].

Although numerous studies have investigated the antibacterial effects of plasma and PDT against *E. faecalis* biofilms, no study has so far compared them. Comparison of different methods can help us to find an effective and efficient way for solving the problem of failure in endodontic treatment. By comparing advantages and disadvantages, one can select and use a method with the highest efficiency. Therefore, in the present work, both plasma jet and PDT were selected to explore the disinfection of *E. faecalis* and compared with each other for the first time.

2. Materials and methods

2.1. Preparation of specimens

One hundred adult single-rooted human teeth with a recently extracted square root (no treatment) were selected, sterilized with 5.25% hypochlorite sodium solution and kept in physiological saline until the time of the experiment at ambient temperature. The crowns of teeth were cut by a rotating diamond saw and the length of all specimens was standardized to 12 mm. The patency of apical foramina was denoted using a size 10 k-file (Dentsply/Maillefer /Tulsa/OK). The working length was established as 0.5 mm less than the total length. The root canals were cleaned and shaped with hand files up to size #40 via the crown-down technique. At each change of instrument size, root canals were irrigated with 2.25% hypochlorite sodium. Subsequently, in order to remove the smear layer from canals and open tubules, the specimens were placed in an ultrasonic bath in EDTA17% and 5.25% sodium hypochlorite solution for 4 min, respectively. Also, apical foramina were sealed by composite resin and finally, all specimens were sterilized by autoclave for 20 min at 121°C (Fig. 1).



Fig. 1. Schematic drawing of the experimental setup.

2.2. Cultivation of *E. faecalis* bacteria

E. faecalis (ATCC9854) was grown on brain-heart infusion (BHI) agar (Merck KgaA, Darmstadt, Germany) plates for 24 h at 37 °C in an aerobic environment. Then, 1 ml of the brain-heart infusion broth (Merck KgaA, Darmstadt, Germany) was inoculated with bacteria by a sterile loop at 37 °C. Afterwards, the bacterium suspension was obtained with the approximate concentration of 10⁸ (CFU/ml) (0.5 McFarland).

2.3. Contamination

Each tooth was transferred to the sterile micro tube. Next, 15 µl of the bacteria suspension was injected into the canals by the sampler. Then, the specimens were incubated for one week. Everyday 15 µl of the sterilized BHI broth was injected into the canal to ensure the survival of bacteria and biofilm formation.

2.4. Light source and photosensitizer

The utilized light source was Dr. Smile diode laser (Lambda, Scientifica, Italy) with a wavelength of 810 nm, output power 0.2 CW that was coupled to an optical fiber with a diameter of 300 µm. The optical fiber was initially placed 1 mm short of the working length, and spiral movements from apical to coronal region were manually performed at a speed of approximately 2 mm/s for 30 s and repeated 3 times with the relaxation time of 5 s between them. The spiral movements were made to allow for effective distribution of the light in all directions of the root canal.

Toluidine blue (Germany, Merck) at a concentration of 0.1 mg/ml was used as the photosensitizer agent in this study. The photosensitizer solution was injected to the teeth with a syringe in a pre-irradiation time of 5 min.

2.5. Plasma reactor

In this study, a dielectric barrier discharge plasma jet was used. It consists of a copper tube as the central electrode and a copper ring as the ground electrode axially aligned. A dielectric barrier (Teflon) was used between two electrodes to reduce the flowing current and prevent electrical discharges. The central electrode was connected to a 25-kHz sinusoidal high-voltage source with a 5-kV peak-to-peak voltage. He and He + 0.5% O₂ were employed as working gases. The flow rate of He and O₂ was 4slm and 20sscm, respectively. The temperature of the plasma plume produced was low and measured by a thermal imaging camera during treatment. The shape and length of plasma flame were appropriate for accessing the root canal. The distance of the plasma jet nozzle from the sample was set at 2 mm (Fig. 2). The produced reactive species in plasma jet were denoted by optical emission spectroscopy 2 cm from the nozzle of the plasma jet.

2.6. Experiment groups

In the first step, 60 teeth were randomly selected and divided into different treatment groups: He, He/O₂ plasma (n = 20) in 5-s groups and PDT (n = 10) in 2-s groups. In the next step, 40 remaining teeth were divided into 4 different disinfection groups (n = 10). All specimens were fixed to the micro plates for the experiment.

Treatment groups in step1

He plasma group: control group (n = 5), 4 min treatment He plasma (n = 5), 6 min treatment He plasma (n = 5), 8 min treatment He plasma (n = 5)

He/O₂ plasma group: control group (n = 5), 4 min treatment He/O₂ plasma (n = 5), 6 min treatment He/O₂ plasma (n = 5), 8 min treatment He/O₂ plasma (n = 5)

PDT group: control group (n = 5), 5 min toluidine blue +

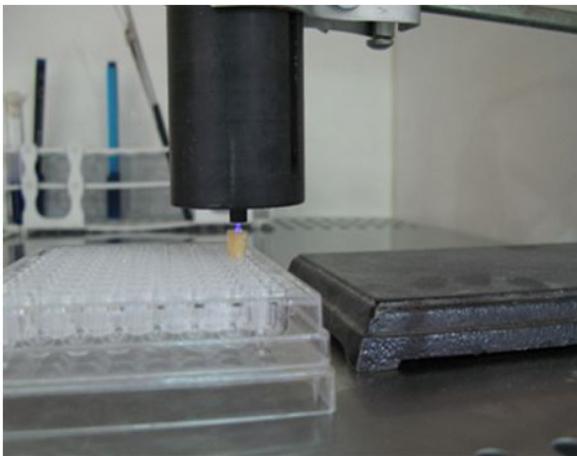


Fig. 2. An image of the plasma jet during root canal treatment.

irradiation for 30 s with 3 repeats (n = 5)

Treatment groups in step 2

“Group 1: control group (n = 10)”

“Group 2: 8 min He plasma treatment (n = 10)”

“Group 3: 8 min He/O₂ plasma treatment (n = 10)”

“Group 4: PDT group: control group (n = 5), 5 min toluidine blue + irradiation for 30 s with 3 repeats (n = 10)”

2.7. Microbiological analysis

Antibacterial effectiveness was investigated using the colony forming unit (CFU) counting method. After all treatments, the root canals were filled with sterile saline normal solution. Two sterile paper points size 40 (were utilized to remove the normal saline solution and bacteria (no biofilm). Sampling was performed using one sterile size 40 k-file for 30 s. The incorporated k-file was transferred into a micro-tube with 1 ml of sterile normal saline solution and vortexed for 20 s. Then, serial decimal dilutions were made and cultured on Petri dishes with BHI agar. The plates were incubated for 48 h at 37 °C. The results were determined by counting CFUs per milliliter.

2.8. Statistical analysis

The GraphPad Prism software (version 6) was employed to analyze the data obtained from the CFU counting analysis. ANOVA and Tukey tests were performed to determine the significant differences between various disinfection methods, and a P value of < 0.05 was considered significant.

2.9. SEM

To examine the structural changes of the *E. faecalis* biofilm before and after treatment, scanning electron microscopy (SEM) was used (Hitachi, SU 3500, Japan). Three groups (control, He/O₂ plasma and PDT) were included in this analysis. In each group, three teeth were randomly selected. Each sample was split longitudinally, mounted on a stub, coated with gold and examined with SEM.

3. Results

3.1. Bactericidal effect

The CFU counting method was applied to study the antibacterial efficacy. After applying 0, 4, 6 and 8 min plasma, the mean values of log CFU/ml were 5.55, 4.53, 4.22 and 3.42 for He plasma (Table 1) and 5.39, 3.91, 2.63 and 0.2 for He/O₂ plasma (Table 2), respectively.

All groups demonstrated a CFU reduction compared with the

Table 1

Log (CFU/ml) of all specimens in treatment with He plasma and Mean values.

8 min	6 min	4min	control	specimen
2.50	4.88	4.80	5.85	1
4.48	4.46	4.15	6.11	2
3.60	4.01	4.54	4.07	3
3.07	3.85	3.37	5.82	4
3.47	3.94	5.38	5.90	5
3.424	4.228	4.53	5.55	Mean

Table 2

Log (CFU/ml) of all specimens in treatment with He/O₂ plasma and their mean values.

8 min	6 min	4min	control	specimen
1	1.98	4.45	5.72	1
0	3.98	4.59	5.73	2
0	2.69	3.01	4.87	3
0	2.58	3.43	5.68	4
0	1.95	4.11	4.97	5
0.2	2.636	3.918	5.394	Mean

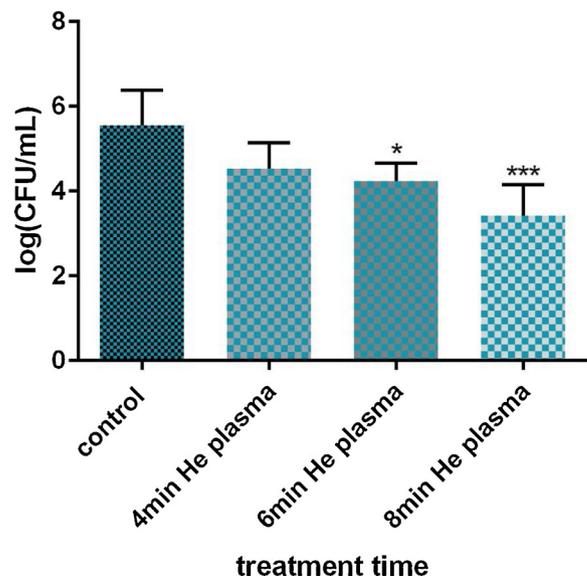


Fig. 3. Disinfection results of He plasma at different times.

control group, and the degree of disinfection increased with increasing plasma treatment time. In the He plasma treatment group, all groups showed at least one log unit reduction, but only 8 min treatment indicated a significant CFU reduction (Fig. 3).

The He/O₂ plasma treatment group demonstrated better results regarding the reduction of CFUs than the He plasma treatment group as shown in the study of performed by Chen et al [25]. All He/O₂ plasma-treated groups indicated a significant CFU reduction (Fig. 4).

The results of PDT, 5 min toluidine blue + irradiation for 30 s with three repeats and relaxation time of 5 s are presented in Fig. 5. The mean value of log CFU/ml was reduced from 5.68 (control group) to 2.49 (Table 3), indicating a significant reduction (p < 0.05) (Fig. 5).

Table 4 and Fig. 6 present the results of the bactericidal effects of He and He/O₂ plasmas for 8 min and PDT. Compared to the control group, all disinfection methods demonstrated a significant CFU reduction (p < 0.05). He/O₂ plasma was the most effective disinfection method among the applied methods. No significant difference was observed between He plasma and PDT.

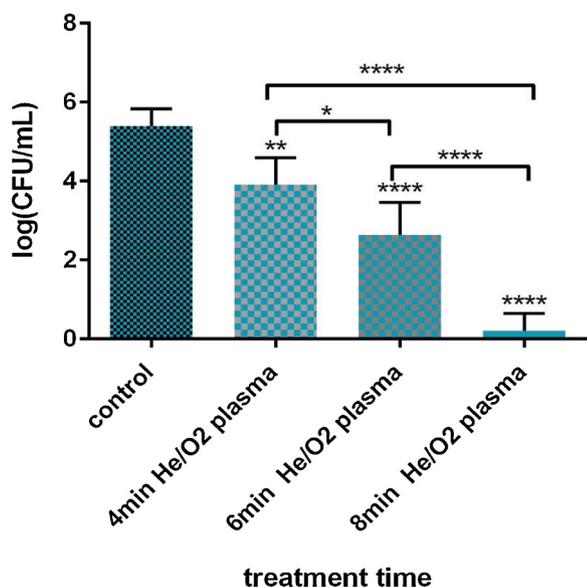


Fig. 4. Disinfection results of He/O₂ plasma at different times.

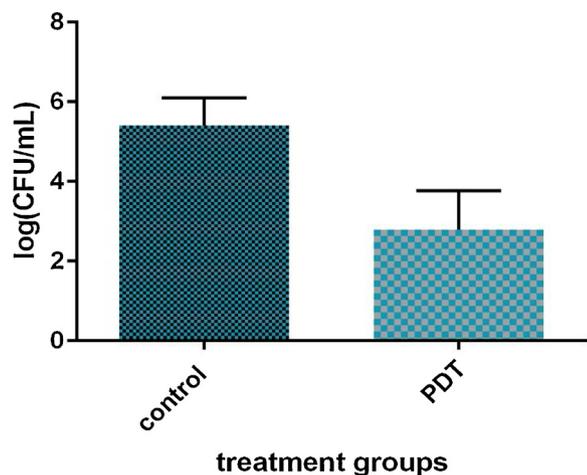


Fig. 5. Disinfection results of PDT (5 min toluidine blue + irradiation for 30 s with three repeats and relaxation time of 5 s).

Table 3
Log (CFU/ml) of all specimens in treatment with PDT and their Mean ± SEM values.

Specimen	control	PDT
1	6.23	3.23
2	6.37	2.83
3	5.15	1.44
4	4.57	3.27
5	5.64	3.51
6	5.9	3.43
7	4.94	1.14
8	5.26	2.75
9	4.28	2.13
10	5.77	4.23
Mean ± SEM	5.411 ± 0.2188	2.796 ± 0.3057

3.2. Optical emission spectra

To examine the bacterial inactivation in plasma treatment, the optical emission spectra of He and He/O₂ at the wavelengths of 100–1100 nm are presented in Fig. 7. The inactivation effect on bacteria by ultraviolet radiation is mostly related to the DNA/RNA damage in UV-C

Table 4
Log (CFU/ml) of all specimens in treatment with He, He/O₂ and PDT and their Mean values.

Specimen	control	PDT	He plasma (8 min)	He plasma (8 min)
1	4.1	2.08	2.45	0
2	4.62	0.98	0.93	0
3	4.81	3.96	3.51	0
4	5.5	1.25	4.02	0.75
5	5.84	2.51	1.98	0
6	4	2.12	2.88	0
7	4.9	1.53	3.16	0
8	6.11	2.97	1.95	0
9	5.35	2.7	3.05	1
10	5.18	1.47	0.96	0
Mean	5.041	2.156	2.489	0.17

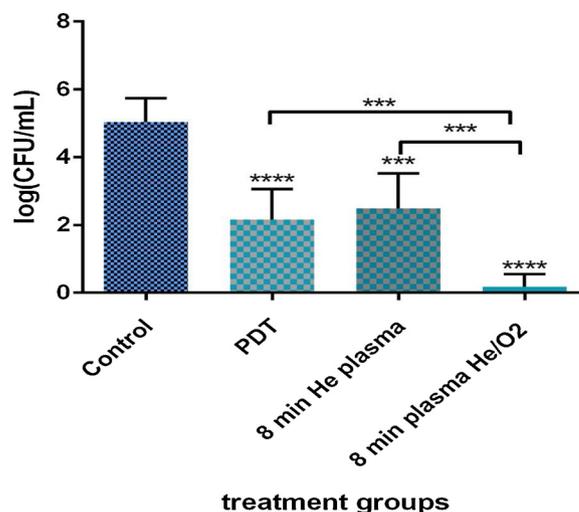


Fig. 6. Disinfection results of different treatment groups: He plasma, He/O₂ plasma (in treatment) and PDT.

(200–280 nm) [28]. However, in the present figure, no significant peak was observed in this range. Consequently, UV does not play a key role in the inactivation of bacteria. The excited O lines at 285 and 777 nm, OH line at 309 nm, molecular nitrogen lines at 316, 337, 357, 380, and 391 nm, O⁺ line at 427 nm, H_α line at 656 nm and excited He atom lines at 501, 587, 640, 669, 707, and 729 nm are clearly found in Fig. 7. According to Fig. 7.B, addition of oxygen to He plasma decreases He emission while enhancing the intensity of reactive oxygen radicals which react with the outer membrane of the microorganism and oxidize them [34–36].

3.3. SEM

The SEM images of split root canals are presented in Fig. 8. The images of scanning electron microscopy clearly confirmed the results of CFU analysis. These images following PDT and plasma treatment demonstrated reduced size, rupture and damaged structure of *E. faecalis* biofilm, compared to the control group.

In the control group, the root canal surface was completely covered by a thick and proximate biofilm, as depicted in Fig. 8A. After PDT, the biofilm structure was broken and replaced with separated bacteria. In this treatment group, the openings of dentinal tubules almost were detected (Fig. 8B) and, in the He/O₂ plasma group, after 8 min treatment, it was observed that the biofilm on the surface of root canal was disappeared and the openings of dentinal tubules were completely detected (Fig. 8C). Similarly numerous studies have examined, cell destruction in plasma treatment, reporting that bacterial cell structures were completely destroyed and the bacteria were completely killed

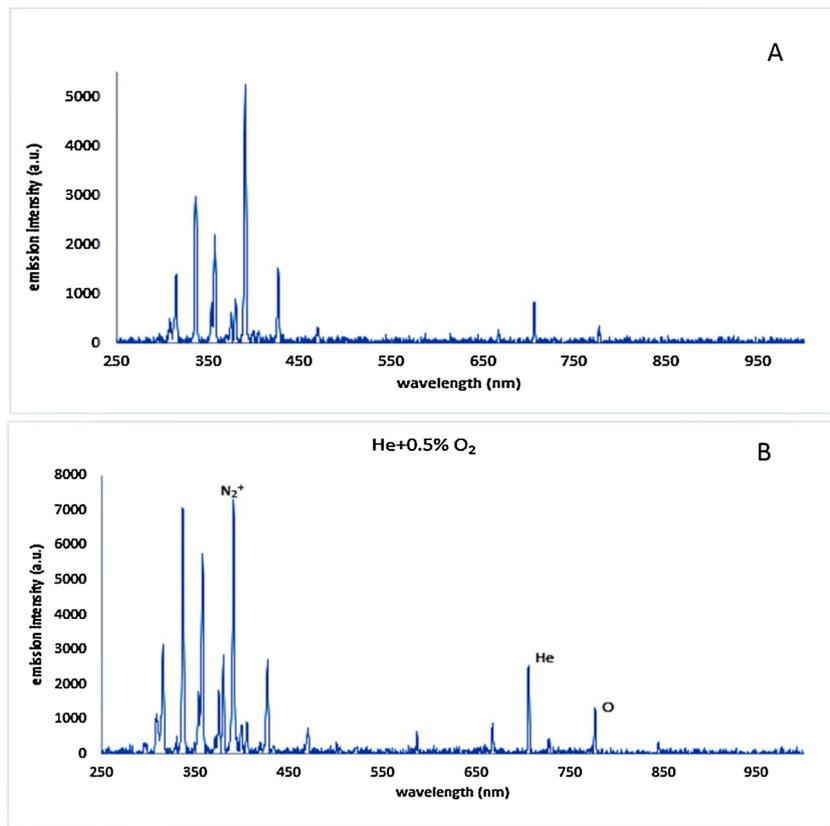


Fig. 7. The optical emission spectra of (A) He and (B) He/O₂.

[24,37].

3.4. Measurement of temperature

The thermal effects of plasma should be considered as an important

parameter due to the sensitivity of living cells. Therefore, the temperature of plasma was recorded by a thermal imaging camera (FLIR 8 with thermal sensitivity < 0.06 °C and Temperature Range: -20 to 250 °C) during the treatment. As seen in Fig. 9, the plasma temperature was less than 40 °C after 4, 6 and 8-min plasma exposure. Therefore,

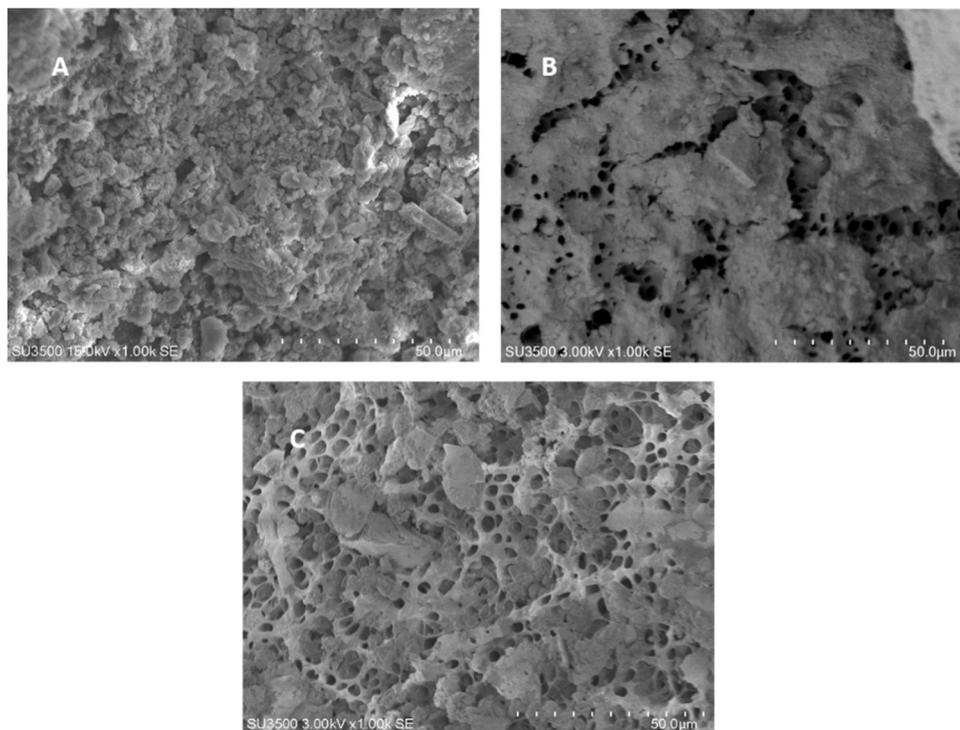


Fig. 8. SEM images of different treatment groups (A) control group (B) PDT treatment and (C) He/O₂ plasma treatment.

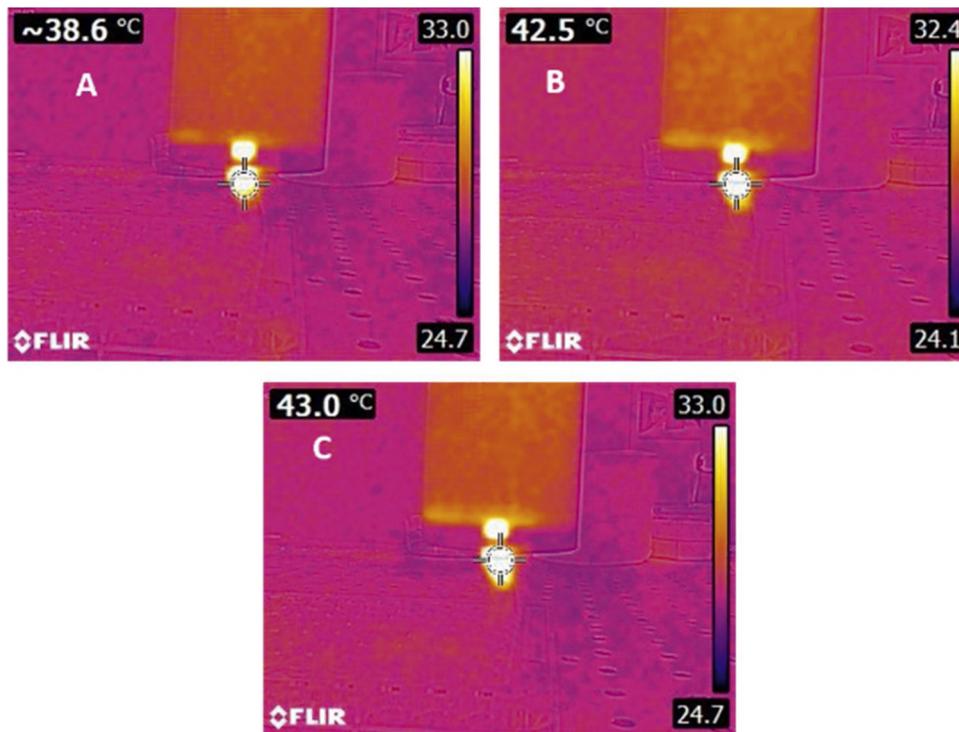


Fig. 9. Thermal camera images of plasma after (A) 4 min (B) 6 min (C) 8 min treatments.

heat had no role in disinfection by plasma. Furthermore, in applying this plasma jet, plasma had no thermal effect such as ablation or coagulation [24,34,37], hence it will be safe for biomedical applications such as root canal therapy.

4. Discussion

Although traditional treatments can significantly reduce the population of bacteria inside the infected root canal but elimination of all bacteria from the root canal is not always achievable by them and bacteria often remain in the root canal. The remaining bacteria can grow again and lead to treatment failure. To overcome this issue, further studies are required.

Researchers have recently introduced new methods including plasma and PDT that have shown promising results in disinfection of root canal.

One of the main bacteria which has a significant role in endodontic failure and root canal infection is *Enterococcus faecalis*. This bacteria has a high affinity for biofilm formation and bacteria in the biofilm form become 1000-fold more resistant to antibacterial agents compared to its planktonic form, hence *E. faecalis* was chosen as the test bacteria in this study.

The aim of this study was to evaluate and compare the effects of two plasmas (He and He/O₂ plasma) at a 5-kV peak-to-peak voltage and PDT with diode laser 810 nm and toluidine blue in disinfecting root canals contaminated with *E. faecalis*.

The antibacterial effects of the plasma jet and PDT resulted from the reactive oxygen species, such as atomic oxygen and free radicals. These species play a main role in the disinfection process because they are able to penetrate the cells and damage essential cellular molecules. In this study, stronger emission lines of reactive oxygen species were detected in He/O₂ plasma.

In this study all groups demonstrated a CFU reduction compared to the control group. In the same treatment conditions, He/O₂ plasma was more effective than He plasma, due to the presence of more ROS in He/O₂ plasma. He plasma and PDT showed the same results. Compared to PDT, He/O₂ plasma was a more effective method. It can be explained by

the gas state of plasma, as it can be directly inserted into the root canal and its junctions, interacts with the biofilm, and destroys it. Another aspect to enhance the effect of plasma is the treatment time, which can be easily extended. It was observed that, when treatment time was extended from 4 to 8 min, CFU was reduced to zero.

To examine the structural changes of the *E. faecalis* biofilm before and after treatment, scanning electron microscopy (SEM) was used. The results of SEM demonstrated reduced size, viability, rupture and damaged structure of *E. faecalis* biofilm, compared to the control group. Heat had no role in disinfection by plasma because the temperature of plasma was less than 40 °C.

The antibacterial effects depend not only on the applied method, but also on inter-individual differences in the anatomy. In this study, we used single-rooted anterior teeth with straight canal. Thus, further studies should be performed to examine the bactericidal effects of the plasma and PDT in multi-jointed and curved root canal.

In conclusion the results of this study revealed that the applied methods had high cytotoxic effects against *E. faecalis* as previously reported. He/O₂ plasma was the most effective disinfection method among the applied methods. PDT and He plasma showed the same results. The bactericidal effects of He/O₂ plasma was promising and could eradicate the microorganisms that may not be killed by routine techniques.

References

- [1] U. Sjögren, D. Figdor, S. Persson, G. Sundqvist, Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis, *Int. Endod. J.* 30 (1997) 297–306, <https://doi.org/10.1111/j.1365-2591.1997.tb00714.x>.
- [2] J.L. Saunders, P.D. Eleazer, P. Zhang, S. Michalek, Effect of a Separated Instrument on Bacterial Penetration of Obturated Root Canals, (2004), pp. 8–10.
- [3] C.H. Stuart, S.A. Schwartz, T.J. Beeson, C.B. Owatz, *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment, *J. Endod.* 32 (2006) 93–98, <https://doi.org/10.1016/j.joen.2005.10.049>.
- [4] J.F. Siqueira Jr., I.N. Rôças, S.S. Paiva, T. Guimarães-Pinto, K.M. Magalhães, K.C. Lima, Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 104 (2007) 122–130, <https://doi.org/10.1016/j.tripleo.2007.01.027>.

- [5] C. Oh, Reduction of Intracanal Bacteria Using GT Rotary vol. 31, (2005), pp. 359–363.
- [6] O. Microbiology, The Antibacterial Action of Sodium Hypochlorite and EDTA in 60 Cases of Endodontic Therapy, (1985).
- [7] J.F. Siqueira Jr., S.S. Paiva, I.N. Rôças, Reduction in the cultivable bacterial populations in infected root canals by a chlorhexidine-based antimicrobial protocol, *J. Endod.* 33 (2007) 541–547, <https://doi.org/10.1016/j.joen.2007.01.008>.
- [8] R.C. Identification, Identification and Antimicrobial Susceptibility of Enterococci Isolated From the Root Canal, (2000), pp. 309–312.
- [9] D. Figdor, The Antimicrobial Effect of Calcium Hydroxide As a Short-term Intracanal Dressing, (1985), pp. 119–125.
- [10] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Bacterial biofilms: a common cause of persistent infections, *Science* 284 (1999) 1318–1322.
- [11] R.M. Love, *Enterococcus faecalis* – A Mechanism for Its Role in Endodontic Failure, (2001), pp. 399–405.
- [12] C.F. Murad, L.M. Sassone, M. Faveri, Microbial diversity in persistent root canal infections investigated by checkerboard DNA-DNA hybridization, *J. Endod.* (2014) 1–8, <https://doi.org/10.1016/j.joen.2014.02.010>.
- [13] L.C. De Paz, Redefining the persistent infection in root canals: possible role of biofilm communities, *J. Endod.* 33 (2007), <https://doi.org/10.1016/j.joen.2006.11.004>.
- [14] I. Portenier, T.W. Dds, D.Ø. Dds, The susceptibility of starved, stationary phase, and growing cells of *Enterococcus faecalis* to endodontic, *J. Endod.* 31 (2005) 380–386.
- [15] R.P. Roach, J.F. Hatton, M.J. Gillespie, Prevention of the ingress of a known virulent bacterium into the root canal system by intracanal medications, *J. Endod.* (2001) 9–12.
- [16] P.S. Stewart, J.W. Costerton, Antibiotic resistance of bacteria in biofilms, *Lancet* 358 (2001) 135–138.
- [17] Aline Chiodi Borges, ATIVIDADE ANTIFÚNGICA E CITOTOXICIDADE DO JATO DE PLASMA FRIO SOB PRESSÃO ATMOSFÉRICA, (2016).
- [18] Z. Lim, J.L. Cheng, T.W. Lim, E.G. Teo, J. Wong, S. George, A. Kishen, Light activated disinfection: an alternative endodontic disinfection strategy, *Aust. Dent. J.* (2009) 108–114, <https://doi.org/10.1111/j.1834-7819.2009.01102.x>.
- [19] R. Wang, H. Zhou, P. Sun, H. Wu, J. Pan, The effect of an atmospheric pressure, DC nonthermal plasma microjet on tooth root canal, dentinal tubules infection and reinfection prevention, *Plasma Med.* 1 (2011) 143–155.
- [20] S.H. Nam, H.W. Lee, S.H. Cho, J.K. Lee, Y.C. Jeon, G.C. Kim, High-efficiency tooth bleaching using non-thermal atmospheric pressure plasma with low concentration of hydrogen peroxide, *J. Appl. Oral Sci.* 21 (2013) 265–270.
- [21] B. Yang, J. Chen, Q. Yu, H. Li, M. Lin, A. Mustapha, L. Hong, Y. Wang, Oral bacterial deactivation using a low-temperature atmospheric argon plasma brush, *J. Dent.* 39 (2011) 48–56, <https://doi.org/10.1016/j.jdent.2010.10.002>.
- [22] L. Canullo, D. Peñarrocha, M. Clementini, G. Iannello, C. Micarelli, Impact of plasma of argon cleaning treatment on implant abutments in patients with a history of periodontal disease and thin biotype: radiographic results at 24-month follow-up of a RCT, *Clin. Oral Implants Res.* 26 (2015) 8–14, <https://doi.org/10.1111/clr.12290>.
- [23] A.G. Whittaker, E.M. Graham, R.L. Baxter, A.C. Jones, P.R. Richardson, G. Meek, G.A. Campbell, A. Aitken, H.C. Baxter, Plasma Cleaning of Dental Instruments, (2004), pp. 37–41, <https://doi.org/10.1016/j.jhin.2003.09.019>.
- [24] J. Pan, K. Sun, Y. Liang, P. Sun, X. Yang, J. Wang, J. Zhang, W. Zhu, J. Fang, Cold plasma therapy of a tooth root canal infected with *Enterococcus faecalis* biofilms in vitro, *J. Endod.* 39 (2013) 105–110, <https://doi.org/10.1016/j.joen.2012.08.017>.
- [25] W. Chen, J. Huang, N. Du, X. Liu, X. Wang, Treatment of *Enterococcus faecalis* Bacteria by a Helium Atmospheric Cold Plasma Brush With Oxygen Addition Treatment of *Enterococcus faecalis* Bacteria by a Helium Atmospheric Cold Plasma Brush With Oxygen Addition, 013304 (2013), pp. 1–5, <https://doi.org/10.1063/1.4732135>.
- [26] L. Oksuz, B. Ureyen, A.D. Kececi, F. Bozduvan, T. Ozt, Efficacy of endodontic applications of ozone and low-temperature atmospheric pressure plasma on root canals infected with *Enterococcus faecalis*, *Lett. Appl. Microbiol.* (2013) 8–15, <https://doi.org/10.1111/lam.12148>.
- [27] O. Victor, P. Denardin, D. Ph, A. Rapoport, D. Ph, R.A. Dedivitis, D. Ph, Photodynamic Therapy for Root Canals Infected with *Enterococcus faecalis* vol. 26, (2008), pp. 209–213, <https://doi.org/10.1089/pho.2007.2124>.
- [28] J.P. Chen, L. Yang, L.K. Wang, B. Zhang, Ultraviolet Radiation for Disinfection, Advanced Physicochemical Treatment Processes, Humana Press Inc, 2006, pp. 317–366.
- [29] L. Bergmans, P. Moisiadis, B. Huybrechts, B. Van Meerbeek, M. Quirynen, Effect of Photo-activated Disinfection on Endodontic Pathogens Ex Vivo, (2008), pp. 227–239, <https://doi.org/10.1111/j.1365-2591.2007.01344.x>.
- [30] J.L. Fimple, C.R. Fontana, F. Foschi, Photodynamic treatment of endodontic polymicrobial infection in vitro, *J. Endod.* 34 (2008), <https://doi.org/10.1016/j.joen.2008.03.011>.
- [31] A.S. Garcez, M.S. Ribeiro, G.P. Tegos, S.C. Nu, A.O.C. Jorge, M.R. Hamblin, Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection, *Lasers Surg. Med.* 66 (2007) 59–66, <https://doi.org/10.1002/lsm.20415>.
- [32] A.S. Garcez, C. Núñez, J.L. Lage-marques, O. Cardoso, Efficiency of NaOCl and laser-assisted photosensitization on the reduction of *Enterococcus faecalis* in vitro, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* (2006) 93–98, <https://doi.org/10.1016/j.tripleo.2006.02.015>.
- [33] J.A. Williams, G.J. Pearson, M. John Colles, Antibacterial action of photoactivated disinfection {PAD} used on endodontic bacteria in planktonic suspension and in artificial and human root canals, *J. Dent.* 34 (2006) 363–371, <https://doi.org/10.1016/j.jdent.2005.08.002>.
- [34] X. Yan, Z. Xiong, F. Zou, S. Zhao, X. Lu, G. Yang, G. He, K. Ostrikov, Plasma-induced death of HepG2 cancer cells: intracellular effects of reactive species, *Plasma Processes and Polymers* 9 (2012) 59–66.
- [35] D.-X. Liu, M.-Z. Rong, X.-H. Wang, F. Iza, M.G. Kong, P. Bruggeman, Main Species and Physicochemical Processes in Cold Atmospheric-pressure He + O₂ Plasmas, *Plasma Processes and Polymers* 7 (2010) 846–865.
- [36] D.X. Liu, F. Iza, X.H. Wang, M.G. Kong, M.Z. Rong, He + O₂ + H₂O Plasmas as a Source of Reactive Oxygen Species, (2011), pp. 30–32, <https://doi.org/10.1063/1.3592775>.
- [37] B.I. Vitro, Y. Li, K. Sun, G. Ye, Y. Liang, H. Pan, Evaluation of cold plasma treatment and safety in disinfecting 3-week root canal *Enterococcus faecalis*, *J. Endod.* (2015) 1–6, <https://doi.org/10.1016/j.joen.2014.10.020>.