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The Antimicrobial Activity of an Atmospheric-Pressure Room-Temperature Plasma in a Simulated Root-Canal Model Infected With *Enterococcus Faecalis*

Xincai Zhou, Zilan Xiong, Yingguang Cao, Xinpei Lu, *Senior Member, IEEE*, and Dexi Liu

Abstract—The antimicrobial activity of an atmospheric-pressure room-temperature plasma jet on simulated root canals infected with *Enterococcus faecalis* is studied. The samples are divided randomly into 12 experimental groups and one control group. All experimental groups exhibited a significant reduction in viable bacteria compared with the control group ($P < 0.01$). The largest reductions were obtained in Group 9 (with plasma jet containing 5.25% sodium hypochlorite sterilization for 12 min after irrigating the root canals with 1-ml sterile physiologic saline) and Group 12 (with plasma-jet sterilization for 12 min after irrigating the root canals with 1-ml sterile physiologic saline), with 6.21 and 5.62 log reductions, respectively. It is concluded that plasma jet containing 5.25% sodium hypochlorite as well as plasma jet only can effectively sterilize the simulated root canals.

Index Terms—Antimicrobial activity, *Enterococcus faecalis*, plasma, root canal.

I. INTRODUCTION

THE *Enterococcus faecalis* is a gram-positive facultative anaerobic bacterium and is the most frequently isolated species, often as a mono-infection, from root canals of endodontically treated teeth with persistent apical periodontitis [1]–[4]. This bacterium is known to survive a wide variety of extreme environmental conditions and has been demonstrated to be resistant to the calcium hydroxide treatment commonly used in the course of endodontic therapy [5]. It can survive for long periods under nutritional deprivation. Sodium hypochlorite and chlorhexidine have been verified to be effective against *E. faecalis* *in vitro*, but they require direct contact with the organism [6].

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Bacteria and their products are major etiologic factors of pulpitis and apical periodontitis [7], [8]. It has also been shown that no periapical inflammation occurs in the absence of bacteria, regardless of the quality of the root-canal filling [9]. Therefore, an important goal of endodontic therapy is the elimination of bacteria from the root canal. Mechanical instrumentation and chemical irrigation, in conjunction with medication of the root-canal system between treatment sessions, can significantly reduce the population of bacteria inside the infected root canal. It is difficult, however, to eradicate all bacteria from the root-canal system [10], [11]. Consequently, the use of an atmospheric-pressure low-temperature plasma device is considered beneficial in efforts to further reduce the number of bacteria and to eradicate infection [12].

In recent years, low-temperature plasmas have emerged as a novel tool for water decontamination, disinfection of medical equipment, implants, blood coagulation, etc. [13]–[25]. Recently, we have reported a room-temperature plasma-jet device that can generate plasma plumes in root canals without causing any harm to human [26]. It has been demonstrated that the plasma jet can efficiently kill *E. faecalis* on agar in petri dish [27], [28]. In this paper, the antimicrobial activity of this device on *E. faecalis* in simulated root canals is studied.

II. MATERIALS AND METHODS

A. Experimental Setup

Photographs of the experimental setup are shown in Fig. 1. Details about the device can be found in [26]. Fig. 1(a) shows the photograph of the plasma jet inside the simulated root-canal model infected with *E. faecalis*, with working gases flowing through 5.25% sodium hypochlorite solution inside a three-neck flask, as shown in Fig. 1(b). The simulated root-canal model is made of resin, with length of 10 mm, width of 10 mm, and height of 30 mm. The length of the inner root canal is about 17 mm, and the diameters of the root-canal orifice and the root tip are 0.7 and 0.4 mm, respectively. The inner diameter of the syringe nozzle is about 1.0 mm. When the simulated root-canal models are treated, the syringe nozzle is vertically put on the root-canal orifice. The working gases used in all the experiments are He/O₂ (1%) with a flow rate of 2 L/min. All the experiments in this paper use the same applied voltage V_a of 8 kV, frequency of 8 kHz, and pulsewidth of 1600 ns.

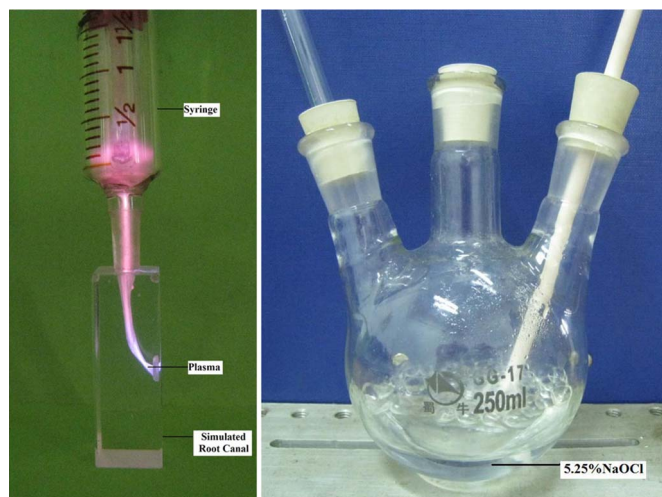


Fig. 1. (a) Photograph of the plasma jet with working gases flowing through 5.25% sodium hypochlorite curving in a simulated root canal infected by *E. faecalis*. (b) Photograph of working gas flowing through 5.25% sodium hypochlorite by using a three-neck flask.

B. Microbial Preparation

A pure culture of *E. faecalis* ATCC 29212 was grown in Mueller–Hinton (M–H) agar and incubated overnight at 37 °C. The bacteria were then inoculated into a test tube containing 2-ml sterile physiological saline. The suspension was adjusted to a turbidity of 6.0×10^8 colony forming unit (CFU)/ml. Finally, the same amount of brain heart infusion broth was added to the test tube. At this point, the concentration of bacteria was 3.0×10^8 CFU/ml (equivalent to 1.0 McFarland unit).

C. Root-Canal Preparation

One hundred and four single-rooted plastic resin blocks (Dentsply Maillefer) were instrumented by an experienced endodontist, using K-files (Dentsply Maillefer) from size 15 to 40 (International Organization for Standardization) with the step-back technique. The blocks were placed in an ultrasonic bath in 17% ethylenediamine tetraacetic acid for 4 min, followed by 5.25% sodium hypochlorite for another 4 min to remove the smear layer. The roots were rinsed with sterile water and placed in an ultrasonic cleaner for 20 min. All plastic blocks were autoclaved for 30 min at 121 °C. Then, all apical foramina of the simulated root canals were sealed with parafilm. Next, 10 ml of bacterial suspension was introduced into each root canal using a sterile micropipette. Afterward, the root-canal orifices were enclosed with parafilm again. Then, all root canals were incubated aerobically at 37 °C for 72 h. Finally, the simulated root canals were blotted dry with size-40 sterile paper points after incubation, and the blocks were divided randomly into six experimental groups of eight blocks each, and one control group of eight blocks, according to different times and different methods of plasma sterilization. The treatment conditions of each group are shown in Table I.

After treatment, each simulated root canal was prepared manually by an experienced endodontist with a new sterile size-45 K-file, and each root canal was filed 20 times. The debris on each file and the wall of each root canal were irrigated with

1 ml of sterile physiological saline from the apical foramen to the root-canal orifice after removing the parafilm, and the debris was collected in a test tube. All test tubes were shaken up. Serial dilutions of all samples were plated onto M–H agar, and the plates were incubated at 37 °C for 24 h. The number of CFUs was counted for each sample.

D. Transmission Electron Microscopy

The *E. faecalis* were grown in two M–H agars and incubated for 72 h at 37 °C. The *E. faecalis* in agar plates were treated with plasma-jet sterilization for 0 and 4 min, respectively. The *E. faecalis* were fixed, centrifuged, dehydrated, and embedded. Ultrathin sections were counterstained. Then, they were examined with a transmission electron microscopy (FEI Tecnai G2 12, Netherlands).

E. Statistical Analysis

Cell counts were logarithmically transformed to normalize the data prior to statistical comparison. The Kruskal–Wallis test and Mann–Whitney analysis were used to determine the differences in bactericidal efficacy. The level of significance was set at 0.05.

III. EXPERIMENTAL RESULTS AND DISCUSSIONS

Figs. 2 and 3 show the results of the antibacterial effects of Group 1–Group 6 and Group 7–Group 12, respectively. It clearly shows that the longer the sterilization time is, the better the bactericidal effect becomes in the experimental groups. All experimental groups exhibited a significant reduction in viable bacteria compared with the control group ($P < 0.01$). The largest reductions in CFU of 6.21 and 5.62 log units were obtained in Group 9 and Group 12, respectively. The reduction in Group 9 was significantly greater than that in Group 12, Group 3, and Group 6 ($P < 0.01$). There was a significant reduction in CFU in Group 9 compared with Group 7 and Group 8. Group 12 showed a significant CFU reduction compared with Group 6, Group 10, and Group 11. However, the difference between Group 1 and Group 4 was not statistically significant ($P > 0.05$). Likewise, the difference between Group 2 and Group 5 was not statistically significant ($P > 0.05$).

Transmission electron micrographs of *E. faecalis* show nuclear chromatin condensation, cell-division arrest, and cell-wall rupture (Fig. 4).

We chose the plastic 30° simulated root canals as subjects because the simulated root canals were easily standardized to exclude interference of the dental anatomic complexity that is present in almost all root-canal systems and that *E. faecalis* readily formed biofilms on plastic substrata [29], [30]. The *E. faecalis* is able to form biofilms that help it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis and antimicrobials than nonbiofilm-producing organisms [31]. The potential plasma agents, such as reactive-oxygen species (ROS), ultraviolet (UV) radiation, and charged particles could result in the damage of the biofilms.

TABLE I
TREATMENT CONDITIONS OF EACH GROUP

Group	Treatment Time (min)	gases flowing through 5.25% sodium hypochlorite or not	irrigating root canals with 1 ml sterile physiologic saline or not
1	4	Yes	No
2	8	Yes	No
3	12	Yes	No
4	4	No	No
5	8	No	No
6	12	No	No
7	4	Yes	Yes
8	8	Yes	Yes
9	12	Yes	Yes
10	4	No	Yes
11	8	No	Yes
12	12	No	Yes
13	Control group: working gas (He/O ₂ , 1%) flowing at 2L/min, for 12 min with the plasma off.		

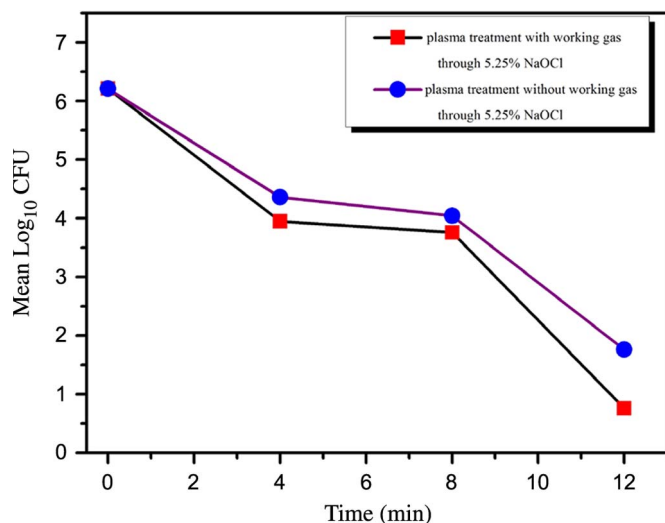


Fig. 2. Plots of viability of the *E. faecalis* after plasma jet with/without working gases flowing through 5.25% sodium hypochlorite sterilization.

Group 9 (with plasma jet with working gases through 5.25% sodium hypochlorite sterilization for 12 min after irrigating the root canals with 1-ml sterile physiologic saline) produced a 6.21 log reduction in bacterial CFU. This result indicates that the *E. faecalis* biofilms were eradicated under this condition.

Irrigation can significantly reduce the population of *E. faecalis* inside the root canal. Therefore, the survival of *E. faecalis* after irrigating the root canals with 1-ml sterile physiologic saline in the experimental groups were far less than the survival of *E. faecalis* without irrigation.

Sodium hypochlorite has shown a high antimicrobial activity against *E. faecalis*. When the working gases flowing through sodium hypochlorite are used, the plasma jet contains hypochlorous acid which easily penetrates the cell membrane aside from ROS and UV. Sodium hypochlorite has a bacteri-

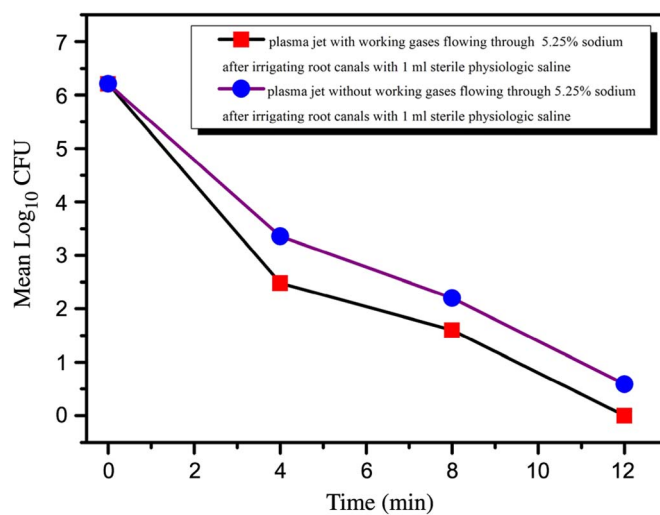


Fig. 3. Plots of viability of the *E. faecalis* after irrigating the root canals with 1-ml sterile physiologic saline and plasma-jet sterilization and the plasma jet with/without working gases flowing through 5.25% sodium hypochlorite sterilization.

riostatic effect, reacting with deoxyribonucleic acid (DNA), ribonucleic acid, and all nucleotides. Hypochlorous acid also has a bactericidal effect, reacting with amino acids to make organic chloramines that are cytotoxic by themselves, with lipids resulting in chlorohydrin. This is also the reason why the plasma jet with gases flowing through the 5.25% sodium hypochlorite has a better bactericidal effect.

It is generally believed that the bactericidal effect of the atmospheric-pressure low-temperature plasma jet is mainly caused by atomic and molecular radicals. It has also been reported that ROS plays an important role in the bactericidal process. In our previous study, we show that the ROS had a significant contribution to the bactericidal process. The ROS has lifetimes of milliseconds or longer, so their concentrations

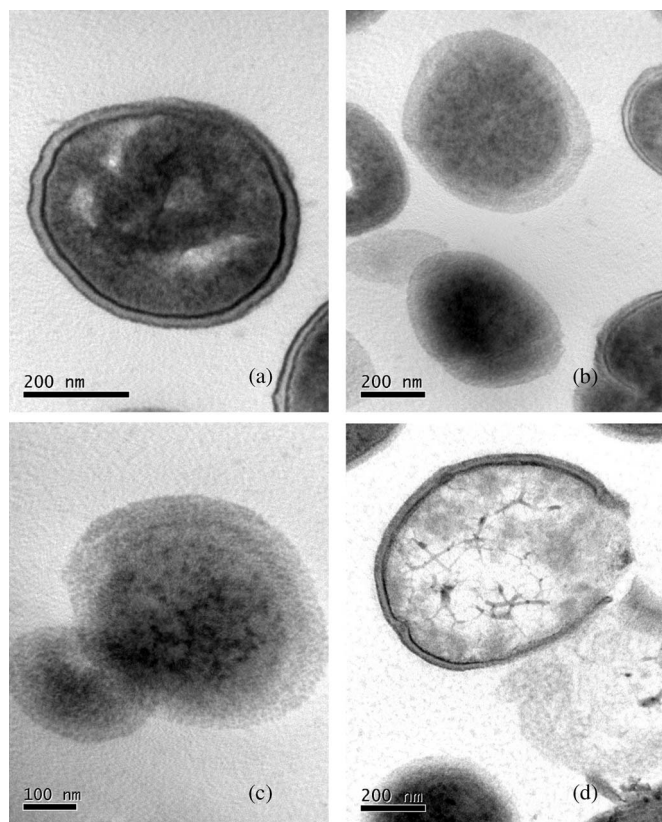


Fig. 4. Transmission electron microscopy of *E. faecalis* after plasma-jet sterilization. (a) Control cell. (b) Nuclear chromatin condensation. (c) Cell-division arrest. (d) Cell-wall rupture.

would not decrease significantly at points that are few centimeters away from the plasma plume [32]. The ROS could penetrate the cells and therefore might induce high levels of DNA damage, which then leads to the induction of apoptosis [33], [34].

The UV intensity emitted by the plasma jet containing 5.25% sodium hypochlorite and the plasma jet is measured. It is around 0.05–0.1 mW/cm². Therefore, UV played a minor role in the sterilization of the bacteria [32]. The plasma plume is at room temperature. Therefore, heat is not responsible for the bactericidal effect.

As the plasma jet could curve within the curved simulated root canal, so the plasma could reach any place inside the inner root canal, killing the bacteria hidden in the places where medicine cannot reach. This is an added advantage for the use of the plasma sterilizer in complicated natural root-canal systems in the future.

In this paper, all the experimental groups have showed a satisfactory bactericidal effect but limited to the simulated root canals. Therefore, further studies should examine the sterilizing effect of the plasma in natural root-canal systems.

IV. CONCLUSION

In conclusion, a simulated root-canal model infected with *E. faecalis* has been treated by an atmospheric-pressure room-temperature plasma jet by using four different kinds of methods. The results show that treatment by plasma jet with working

gases flowing through 5.25% sodium hypochlorite after irrigating the root canals with 1-ml sterile physiologic saline gives the most satisfactory result (killing nearly all the *E. faecalis* in the simulated root-canal model), while other ways also work well. It may be a new way to treat the root canal in clinic; however, further studies on treating natural root-canal systems should be done in the future.

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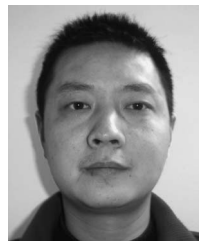


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