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Plasma Sterilization of Root Canal Abscess

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ABSTRACT: We perform *in vitro* validation of two plasma systems to determine whether bacteria inactivation occurs in a root canal abscess during root canal dental surgery. Results indicate that plasma may be a very effective tool for minimally invasive inactivation of bacteria deep inside the tooth, without causing excessive damage to the tooth surface. We show effective inactivation of ~ 10^4 colony forming units of *Enterococcus faecalis* following 10-s treatment by nanosecond-pulsed corona discharge that is ignited using a standard dental tool (Pathfinder®) as the plasma guide.

KEY WORDS: plasma, sterilization, dental surgery, root canal, corona discharge

I. INTRODUCTION

Root canal therapy is used to eliminate the infected pulp of a tooth and protect the decontaminated tooth from future microbial invasion. Each year, more than 25 million root canal treatments are performed globally.[1–4] During this procedure, sodium hypochlorite (NaClO) is routinely used as an antimicrobial agent to irrigate the infected tooth. However, retreatment may be needed due to the occasional persistent infection that may be caused by inefficient disinfection. Previous studies have reported that complications and long-term neurologic injuries can be caused by the NaClO irrigant solution.[5] Other innovative disinfection methods, such as laser and ozone, have also been studied to sterilize a contaminated tooth, but these results were debatable.[6] Therefore, we proposed atmospheric-pressure nonthermal plasma sterilization to be a potential tool for use in dentistry.[7–9] Previous studies have evaluated the application of a plasma jet in tooth root canal disinfection.[9–11] In this article, two novel nonthermal plasma systems, pin-to-hole spark discharge (PHD) and corona discharge, are proposed and their potential use in root canal sterilization validated.

To increase the success rate of root canal therapy, endodontic pathology has been studied. Research has shown that various microorganism species have a role in intraradicular and extraradicular infection. Although most microorganisms can be deactivated with traditional antimicrobial treatments, certain species are resistant to treatment, including some Gram-negative bacteria (e.g., *Fusobacterium nucleatum*, *Campylobacter rectus*, etc.) and various Gram-positive species (e.g., *Streptococcus mitis*, *Olsenella uli*, etc.). Such bacteria can survive and cause persistent infection. Among these micro-

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organisms, *Enterococcus faecalis* has been repeatedly reported as the most commonly identified species in reinfected root canals.[12] Although its prevalence in endodontic infection is debatable,[13] *E. faecalis* is one of the common enteric Gram-positive bacteria and normally inhabits the intestine and oral cavity. Some of its characteristics, such as resistance to several irrigants (e.g., NaClO), ability to form biofilms in medicated canals, and resistance to extreme environments, enable it to survive and proliferate in a treated root canal.[12] Jain et al. showed that *E. faecalis* is able to produce the required proteins to receive multidrug resistance genes from the donor,[14] which makes treatment even more arduous. We thus selected *E. faecalis* as the subject of decontamination to evaluate the two plasma systems more accurately.

II. METHODS AND MATERIALS

A. Root Canal Simulation Block

Figure 1(a) shows a root canal simulation block, where the inactivation of microorganisms is performed through a thin opening at the bottom of the tooth that is marked "area

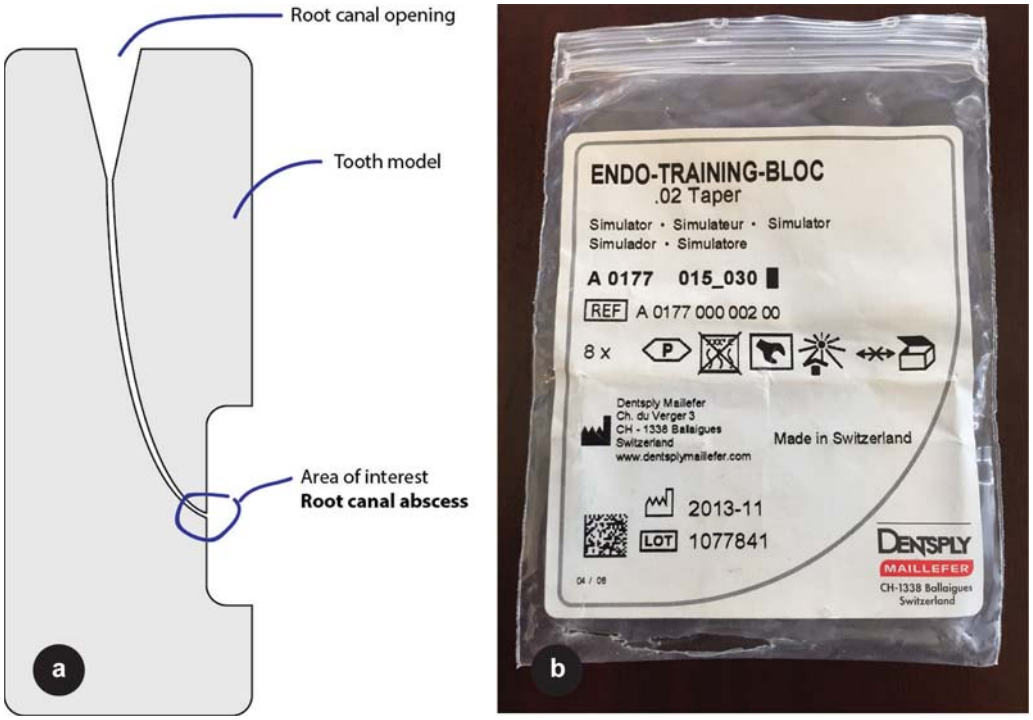

FIG. 1: (a) Schematic of root canal simulation block. Decontamination is performed through the thin opening that is labeled "Area of interest"; (b) a packaged bag of root canal simulation block provides information about the block.

of interest" in the image. More information about the root canal simulation block is displayed in Fig. 1(b).

B. Plasma System I: PHD with MicroProbes

We used a MicroProbes® (Gaithersburg, MD) concentric electrode with a metal shield to deliver plasma into the tooth. It is worth mentioning that for this study, we used a 500-μm electrode, but electrodes as small as 325 μm are also available for use in future experiments. To generate the plasma spark, a high-voltage pulse was delivered to the center electrode, and the outside electrode was grounded. We delivered plasma treatment by inserting the electrode into the root canal cavity. To verify the delivery of active species in the root canal abscess cavity, the cavity was filled with fine powder, as is demonstrated in Fig. 2, and its movement during the discharge operation was recorded (see Fig. 3).

The PHD electrode and power supply were previously described in detail elsewhere.[15] Discharge was ignited by applying high positive potential to the central electrode and grounding the outer electrode. To provide high-discharge energy while keeping average gas temperature low, we powered the electrode system with a capacitor. This resulted in the formation of a dense energetic spark that lasted for ~ 3.5 μs. Due to the low repetition frequency of ~ 7 Hz and the short pulse duration, average gas temperature did not exceed room temperature. PHD plasma characteristics included peak voltage of 3.2 kV, pulse duration of 3.5 μs, frequency of 7 Hz, and energy per pulse of 0.1 J.

Although average gas temperature is low, plasma temperature itself is expected to be relatively high enough to cause production of a significant amount of NO and other

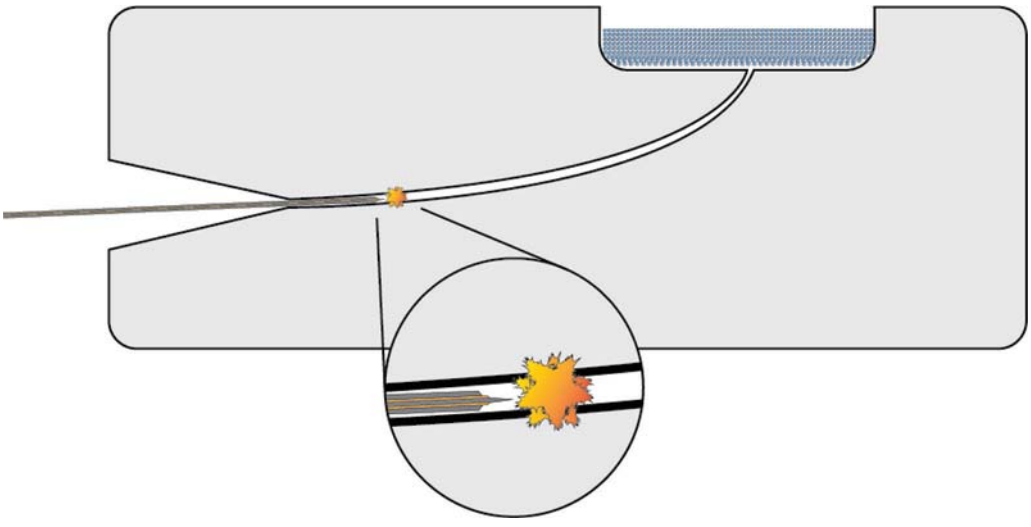

FIG. 2: Active species delivery verification system

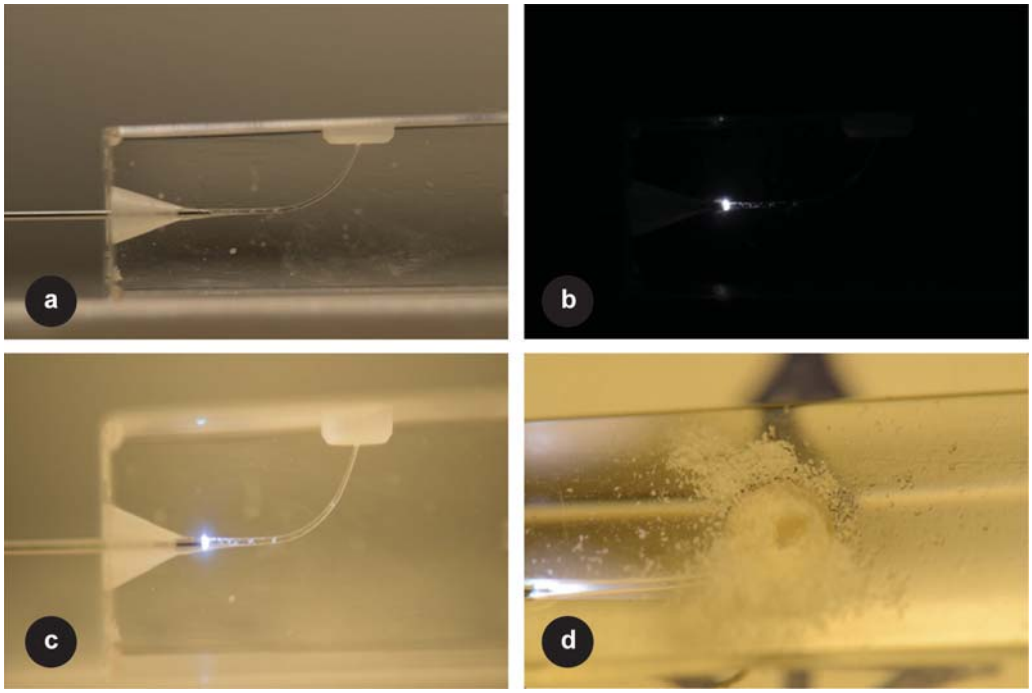

FIG. 3: Photographs of the electrode inserted into the root canal block in (a) lighted room. Spark discharge in the root canal block without (b) and with (c) room lights. (d) Frame from a recorded video showing fine powder "jumping." This indicates that active species–contained flow of plasma afterglow was delivered to the root canal abscess.

reactive species that have been reported to effectively inactivate pathogens.[15,16] As can be seen from the spectrum of radiation reported previously,[15] this discharge radiates intensely in the ultraviolet (UV) range. We measured the total amount of UV light irradiated by plasma using a IL1700 photometer (International Light Technologies; Peabody, MA) to be 5 ± 1 μW/cm^2, which is quite significant for bacterial inactivation, especially in combination with reactive species that were produced in the plasma.[16]

C. Plasma System II: Corona Discharge with Pathfinder Probe

To reach the abscess and generate plasma at the desired location, we used a standard dental Pathfinder® probe (shown in Fig. 4) that is available in dental supply stores. To generate plasma, probes were coated with a thin layer of dielectric material (spray-on acrylic, typically used to coat circuit boards), and then the acrylic from the tip of the Pathfinder probe (~ 0.5 mm) was washed away with acetone. After the process, probes were allowed to dry for 3 h. The coated Pathfinder probe was then inserted into the root canal until the probe tip reached the root canal abscess cavity, as shown in Fig. 5(a) and (b). By applying high voltage on the coated Pathfinder probes and grounding the surface

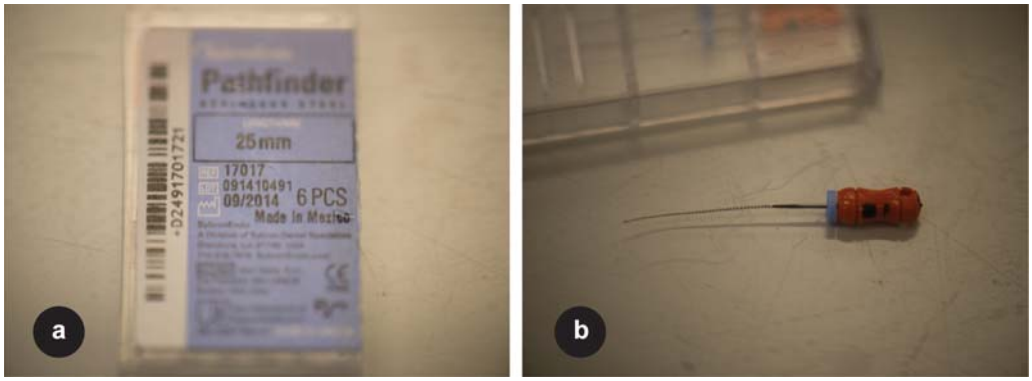

FIG. 4: (a) Standard dental pathfinder probe with related information; (b) standard dental pathfinder probe coated with a thin layer of dielectric material

that was touched by the bottom of the simulation block, corona plasma discharge was generated, as shown in Fig. 5(c).

To initiate the discharge, we used pulses with + 16-kV amplitude in 50-Ω coaxial cable (32 kV on the high-voltage electrode tip caused by pulse reflection), with 10-ns pulse duration (90% amplitude), 0.3-ns rise time, and 3-ns fall time. Maximum pulse frequency was 5 kHz. The generator that we used, purchased from FID GmbH (Burbach,

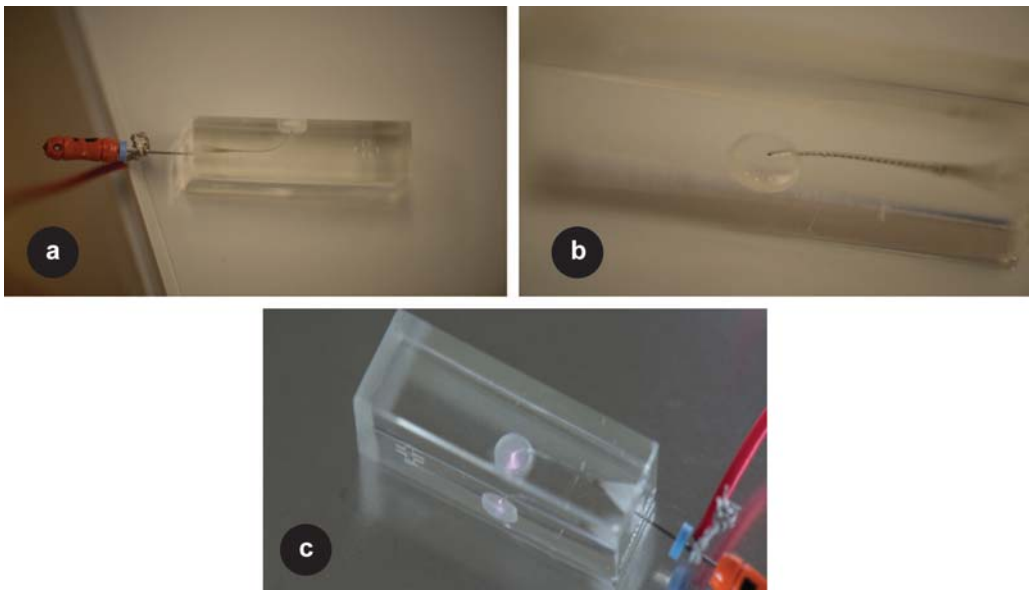

FIG. 5: Photos show (a) root canal block with inserted standard dental pathfinder probe; (b) root canal abscess with pathfinder probe tip, indicating position of the inserted pathfinder probe; (c) corona plasma discharge in root canal abscess cavity. The background shows a grounded metal plate.

Germany), is based on solid-state switches; we have previously reported on the analysis of this discharge system elsewhere.[17,18]

D. Validation System Setup

In both systems, we used bacteria-inoculated blood agar plates and a root canal simulation block to mimic the infected pulp and tooth. We applied PHD and corona discharge plasma for various time spans to investigate their potential application in root canal therapy. *E. faecalis* was inoculated in brain heart infusion broth and incubated overnight. The fresh cultured *E. faecalis* broth was then diluted to 1.5×10^6 colony forming units (CFU)/mL with phosphate-buffered saline to prepare the working bacteria solution. Next, 100 μL of working bacteria solution (1.5×10^6 CFU/mL) was added onto the blood agar plate (5%) and evenly spread using a sterile cell spreader. When the added bacteria solution was totally absorbed by the agar, the contaminated plates were ready to use.

We carefully placed the root canal simulation block onto the surface of the prepared agar plate, and then the MicroProbes® concentric electrode was gently inserted into the simulation block through the opening. This setup is shown in Fig. 6. For corona discharge, the simulation block with the probe inserted was carefully placed onto the inoculated blood agar with its abscess facing downward, which created a cavity connected to the outside only through the opening of the block. This simulated the infected root canal cavity (Fig. 7). By applying high positive potential on the probe and grounding the agar, corona discharge was generated.

E. Treatment and Incubation

For both systems, except for treatment time, treatment conditions among groups remained the same as were described above, and each experiment is repeated in triplicate. For the PHD plasma system, treatment time for each group was 1, 3, 9, 30, and 90 s.

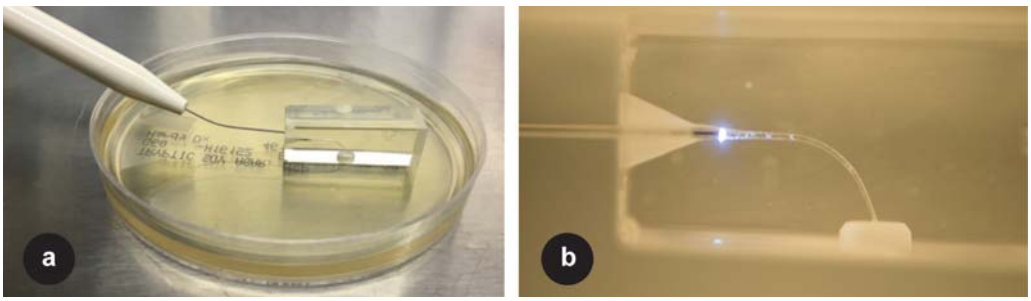

FIG. 6: (a) PHD plasma system setup. Here, a trypticase soy agar plate (TSA) was used to better demonstrate the position of each unit. (b) Photo of spark discharge in the root canal block shows the concentric electrode tip.

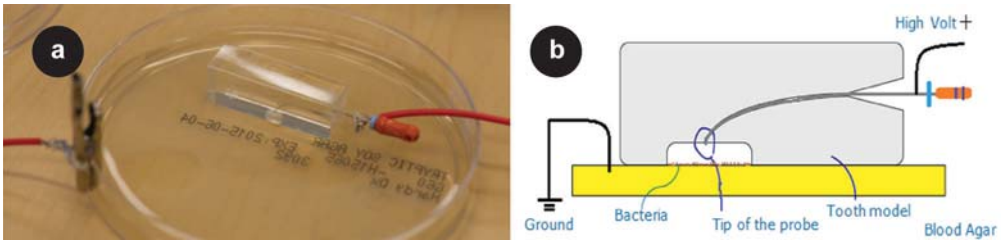

FIG. 7: (a) Corona discharge system setup, in which the TSA plate was used to show the position of each unit; (b) validation setup of corona discharge

For the corona discharge system, treatment time was set as 4, 8, 16, 32, and 110 s, again in triplicate. In both validation systems, after treatment was finished, the block was removed carefully to avoid recontamination of the treated area. All plates were incubated for 16 h at 37°C.

III. RESULTS

A. Results of PHD treatment

We observed generation of plasma bullets and movement of plasma afterglow gas going through the root canal cavity. We measured bacterial inactivation and observed only ~ 80% inactivation in the cavity following 30 s of treatment, an amount that is insufficient for an effective medical procedure.

B. Results of Corona Discharge Treatment

Results and observations of *E. faecalis* treatment with pulsed corona discharge for 4–32 s are discussed below. As can be clearly seen in Fig. 8, bacterial inactivation is observed, with minimal damage to the agar.

IV. DISCUSSION AND CONCLUSIONS

Application of nonthermal plasma in dentistry has been considered previously. Numerous studies have investigated the potential use of a plasma jet in root canal disinfection. In 2004, Sladek et al. studied the feasibility of a He/O_2 plasma jet and showed that *Escherichia coli* (~ 10^8 U/mL) that is spread on agar plates can be reduced by ~ 2 log after 60 s of treatment.[11] A later study by Jiang et al. reported that after 60 s of He/O_2 plasma jet treatment, *Bacillus atrophaeus* (~ 5×10^7 cells/plates), that was spread onto an agar plate, was completely disinfected. The feasibility of the plasma jet has also investigated in an extracted tooth.[19] Lu et al. concluded that after a 10-min treatment of He/O_2 (20%) plasma jet on an extracted tooth, colony count was reduced by 2 log, compared to the untreated group, and disinfection effectiveness of both He/O_2 and Ar/O_2 plasma jets was

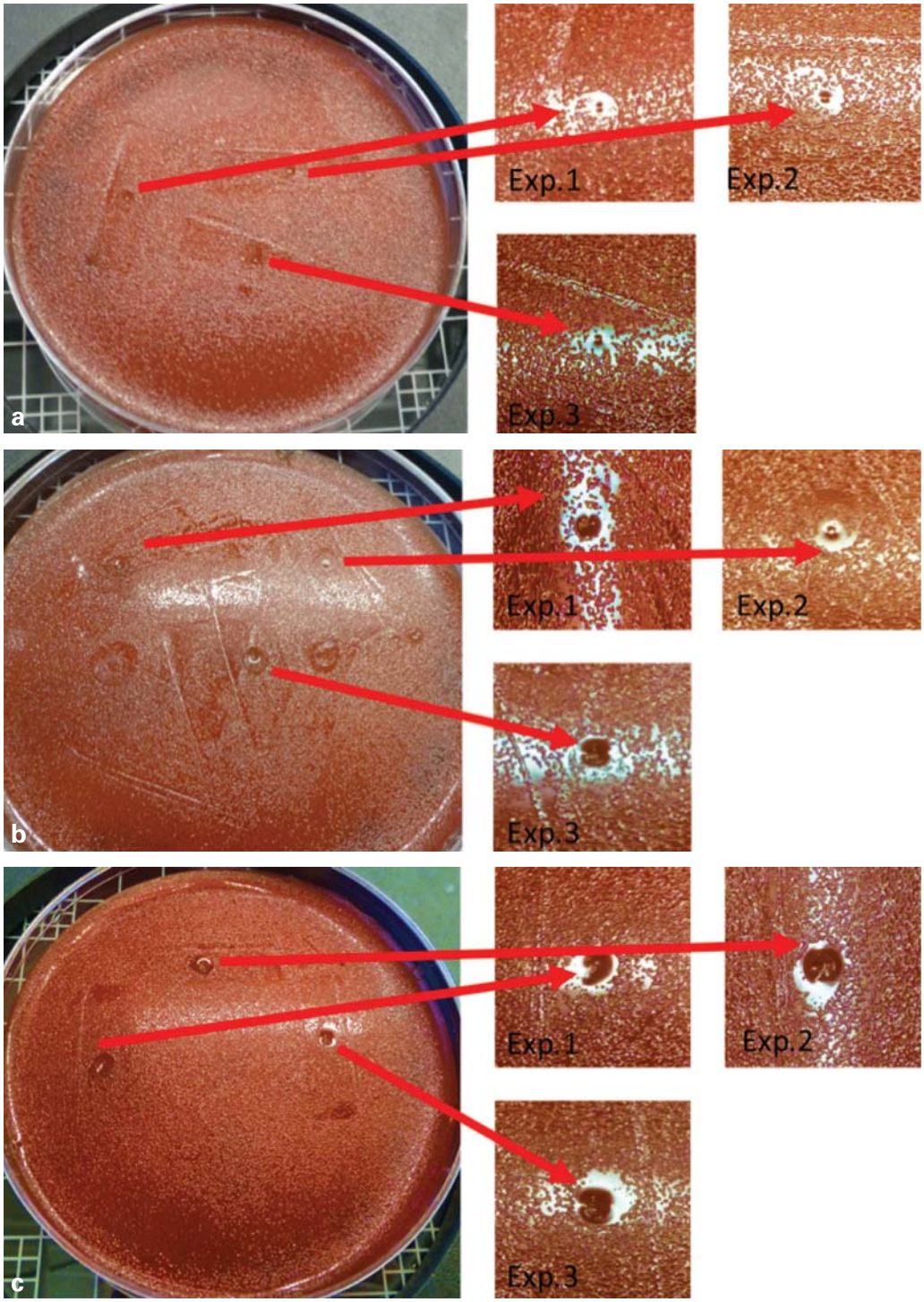

FIG. 8.

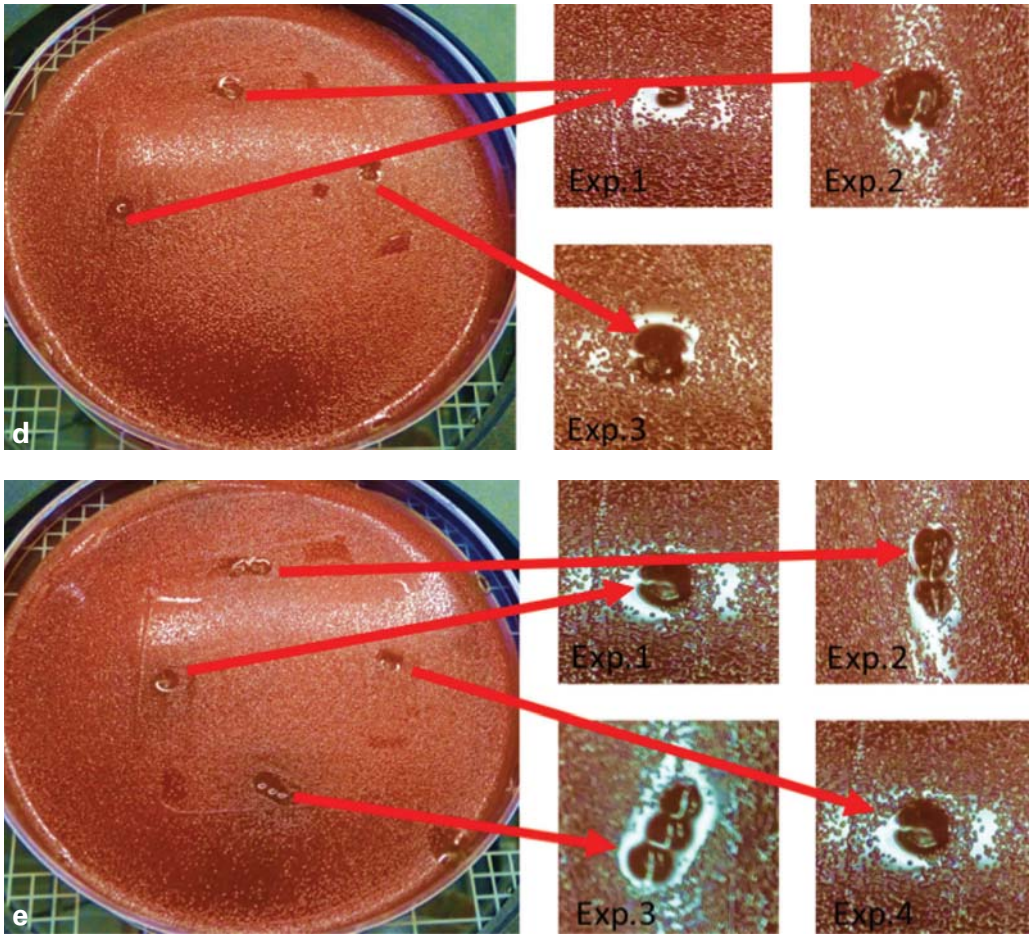

FIG. 8: Results of *E. faecalis* on blood agar plates (1.5×10^5 CFU/plate) after (a) 4-s corona discharge treatment (some bacteria survived, and agar was slightly dried in the treated area); (b) 8-s corona discharge treatment (a few bacteria survived and agar was slightly damaged in the treated area); (c) 16-s corona discharge treatment (no bacteria survived, and agar was significantly dried and damaged in the treated area); (d) 32-s corona discharge treatment (complete bacteria inactivation was achieved, and agar was significantly dried and damaged); (e) three 11-s corona discharge treatments (complete bacteria inactivation was achieved, and agar was significantly dried and damaged).

evaluated to be similar.[20] Later, Wang et al. and Pan et al. studied the deactivation effect of an Ar/O_2 (2%) plasma jet on biofilm in an extracted tooth and reported that complete disinfection could be achieved in 30 and 10 min, respectively.[9,10] Compared to previous studies, we proposed that a corona discharge system can achieve complete inactivation of *E. faecalis* in 16 s by delivering short-pulsed discharge to the inside of a root canal during a dental procedure. The possible explanation of this effectiveness might be di-

rect plasma exposure of bacteria, which allows the short-life active species to reach the bacterial cell, whereas a plasma jet can only deliver long-life molecules. Direct plasma disinfection was already shown to achieve inactivation more quickly than indirect treatment by jet or otherwise.[21] The presented *in vitro* validation is promising, but further *ex vivo* and *in vivo* validations in an animal model are required, especially considering previous studies that imply that biofilm formed inside of the tooth cavity might take longer to completely inactivate.

REFERENCES

1. Zehnder M. Root canal irrigants. J Endodont. 2006;32(5):389–98.
2. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Eur J Oral Sci. 1981;89(4):321–8.
3. Peters OA. Current challenges and concepts in the preparation of root canal systems: A review. J Endodont. 2004;30(8):559-67.
4. Siqueira JF, Rôças IN, Ricucci D. Internal tooth anatomy and root canal instrumentation. In: The root canal anatomy in permanent dentition. Berlin: Springer; 2019. pp. 277–302.
5. Bosch-Aranda ML, Canalda-Sahli C, Figueiredo R, Cosme G-E. Complications following an accidental sodium hypochlorite extrusion: A report of two cases. J Clin Exp Dent. 2012; Jul;4(3):e194–8.
6. Holliday R, Alani A. Traditional and contemporary techniques for optimizing root canal irrigation. Dent Update. 2014;41(1):51–61.
7. Pan J, Sun P, Tian Y, Zhou H, Wu H, Bai N, Liu F, Zhu W, Zhang J, Becker KH, Fang J. A novel method of tooth whitening using cold plasma microjet driven by direct current in atmospheric-pressure air. IEEE Trans Plasma Sci. 2010;38(11):3143–51.
8. Pan J, Sun P, Tian Y, Zhou H, Wu H, Bai N, Wang L, Yu C, Zhang J, Zhu W, Becker KH, Fang J. Tooth whitening with hydrogen peroxide assisted by a direct-current cold atmospheric-pressure air plasma microjet. IEEE Trans Plasma Sci. 2010;38(8):1892–6.
9. Wang R, Zhou H, Sun P, Wu H, Pan J, Zhu W, Zhang J, Fang J. The effect of an atmospheric pressure, DC nonthermal plasma microjet on tooth root canal, dentinal tubules infection and reinfection prevention. Plasma Med. 2011;1(2):143–55.
10. Pan J, Sun K, Liang Y, Sun P, Yang X, Wang J, Zhang J, Zhu W, Fang J, Becker KH. Cold plasma therapy of a tooth root canal infected with Enterococcus faecalis biofilms in vitro. J Endod. 2013;39(1):105–10.
11. Sladek REJ, Stoffels E, Walraven R, Tielbeek RJA, Koolhoven RA. Plasma treatment of dental cavities: A feasibility study. IEEE Trans Plasma Sci. 2004;32(4):1540–3.
12. Narayanan L, Lakshmi, Vaishnavi C. Endodontic microbiology. J Conserv Dent. 2010;13(4):233–9.
13. Murad CF, Sassone LM, Faveri M, Hirata Jr. R, Figueiredo L, Feres M. Microbial diversity in persistent root canal infections investigated by checkerboard DNA–DNA hybridization. J Endod. 2014;40(7):899–906.
14. Jain H, Mulay S, Mullany P. Persistence of endodontic infection and Enterococcus faecalis: Role of horizontal gene transfer. Gene Rep. 2016(5):112–6.
15. Chakravarthy K, Dobrynin D, Fridman G, Friedman G, Murthy S, Fridman AA. Cold spark discharge plasma treatment of inflammatory bowel disease in an animal model of ulcerative colitis. Plasma Med. 2011;1(1):3–19.
16. Dobrynin D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of direct plasma interaction with living tissue. New J Phys. 2009(11):115020.
17. Ayan H, Fridman G, Gutsol AF, Vasilets V, Fridman A, Friedman G. Nanosecond-pulsed uniform dielectric-barrier discharge. IEEE Trans Plasma Sci. 2008;36(2):504–8.

18. Ranieri P, Shrivastav R, Wang M, Lin A, Fridman G, Fridman AA, Han L-H, Miller V. Nanosecond pulsed dielectric barrier discharge induced anti-tumor effects propagate through the depth of tissue via intracellular signaling. Plasma Med. 2017;7(3)283–97.
19. Jiang C, Chen M-Y, Schaudinn C, Gorur A, Vernier PT, Costerton W, Jaramillo DE, Sedghizadeh PP, Gundersen MA. Pulsed atmospheric-pressure cold plasma for endodontic disinfection. IEEE Trans Plasma Sci. 2009;37(7):1190–5.
20. Lu X, Cao Y, Yang P, Xiong Q, Xiong Z, Xian Y, Pan Y. An RC plasma device for sterilization of root canal of teeth. IEEE Trans Plasma Sci. 2009;37(5):668–73.
21. Fridman G, Brooks AD, Balasubramanian M, Fridman A, Gutsol A, Vasilets VN, Ayan H, Friedman G. Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria. Plasma Proc Polymers. 2007;4(4):370–5.