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Effect of Transient Spark Disinfection on Various Endodontics-Relevant Bacteria

L.A. Ledernez,[a,*] F. Engesser,[a] M.J. Altenburger,[b] G.A. Urban,[a] & M.E. Bergmann[a]

[a]Department of Microsystems Engineering (IMTEK), Laboratory for Sensors, University of Freiburg, Freiburg, Germany; [b]Department of Operative Dentistry and Periodontology, Center for Dental Medicine, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

*Address all correspondence to: L.A. Ledernez, Department of Microsystems Engineering (IMTEK), Laboratory for Sensors, University of Freiburg, Freiburg, Germany 79110; Tel.: +49 761 203 7269; Fax: +49 761 203 7262, E-mail: ledernez@imtek.de

ABSTRACT: The *in vitro* efficacy of transient spark disinfection was examined on different types of bacteria that are relevant in endodontics, in particular *Enterococcus faecalis*. A device was constructed so that it could be inserted down to the apex of prepared root canals and could be applied in conventional, regenerative, and reparative endodontics. Starting with a bacterial load of 0.2 mL of undiluted 10^9-bacteria/mL spread on a Petri dish, 3-min treatment led to a 10^6-CFU/cm^2 reduction in bacterial load over a surface area relevant to root canal treatment.

KEY WORDS: plasma, atmospheric pressure, transient spark, tooth, root canal, bacteria, disinfection, *E. faecalis*

I. INTRODUCTION

Atmospheric-pressure plasmas are being used in the medical field to treat, for example, the surfaces of instruments (e.g. sterilization) and implants (e.g. to increase their biocompatibility).[1] The particles constituting the plasma contribute to the sterilization of surfaces by deactivating, killing, or disintegrating microorganisms such as bacteria and viruses.[1] Atmospheric-pressure low-temperature plasma jets are a topic of research into new applications in human medicine—for example, treatment of chronic wounds[2] and cancer therapy.[3,4]

The interest here is the disinfection of tooth root canals in endodontic therapy. The plasma jet offers an alternative to the standard NaOCl solution, which comes with major drawbacks, such as very limited penetration of the apical third of the root canal and dentinal tubules.[5] To overcome those drawbacks, clinicians apply pressure while injecting a sodium hypochlorite (NaOCl) solution into the root canal with or without supplemental ultrasonic activation to decrease its surface tension. Overpressing, however, leads to an extrusion of the liquid beyond the apical foramen with considerable adverse effects, such as facial ecchymosis and mucosal and bone necrosis, with sometimes lasting consequences.[6–8]

Typical NaOCl concentrations are cytotoxic and may result in hemolyses.[9,10] Even at a low concentration (< 0.5%), NaOCl leads to acute inflammation and necrosis of vital tissue.[11] Clinicians must therefore strike a balance between ensuring the success of the root canal treatment (pressing the irrigation solution deeper in the canal) and

decreasing the risk of adverse events (not overpressing). Overall, about 15% to 32% of all root canal treatments are clinically insufficient, leading to reinfection by the remaining bacteria.[12] Moreover, NaOCl changes the properties and characteristics of dentin (e.g., strength, microhardness), influencing the outcome of treatment (e.g., mechanical stability of the tooth) and post-treatment processes in the tooth.[13]

It should come as no surprise that alternatives to sodium hypochlorite are being explored. Cold atmospheric-pressure plasmas, proven to be efficient at killing bacteria,[14,15] offer one such alternative. Various atmospheric-pressure plasma devices have been tested on bacteria and bacterial biofilms on Petri dishes and in root canals.

The plasma jet device (NTP Plume) presented by Schaudinn et al. showed low efficacy in eliminating biofilms inside a root canal even with 30-min treatment. The researchers concluded that, because the plasma jet is efficient only over short distances, its nozzle needs to be flexible and inserted so that the plasma can impinge on the bacteria.[16]

Üreyen Kaya et al. evaluated the antibacterial efficacy of another plasma jet on *Enterococcus faecalis* in root canals. Compared to NaOCl, this one showed a higher efficacy in the middle third of the canal but a similar efficacy in the coronal and apical thirds.[17]

Jiang et al. reported a 10^3-CFU/cm^2 bacterial reduction in *Bacillus atrophaeus* on agar plates after 5-min treatment with a "plasma dental probe." Tested in a root canal, the treatment showed relatively good efficacy in the upper half of the tooth but did not have an effect in the lower half, which the plasma failed to reach.[18]

Wang et al. applied a plasma microjet to single-rooted teeth contaminated with a 10^6-CFU/mL suspension of *E. faecalis*. Based on SEM images, they concluded that 8-min treatment partially cleaned the canal. Treatment of 30 min was required to prevent reinfection of the canal after 7 days.[19]

Ballout et al. compared the bactericidal efficacy inside root canals of two plasma devices (kINPen MED and PlasmaDerm) with that of NaOCl. The PlasmaDerm showed no effect; the kINPen MED showed little effect in the coronal region and nearly no effect in the apical region. The researchers noted that, PlasmaDerm being a dielectric barrier discharge (DBD) device, the plasma generated by it did not penetrate the canal.[20] The kINPen MED was designed for dermatology applications and, because it does not have a nozzle that can be inserted inside a root canal, the jet penetrates only partially.

Lu et al. developed a single-electrode "RC plasma device." The nozzle is not flexible but has a diameter of about 0.7 mm and can therefore be inserted into the root canal to about 1 cm. Tested with *E. faecalis* on Petri dishes, 4-min treatment with the device showed good qualitative results.[21] However, the device has not been tested in teeth.

The plasma devices tested in root canals thus far have one main drawback in common: the generated plasma does not penetrate deeply enough to be efficient where it really counts: at the apex of the tooth. Long treatment times (> 30 min) may partially overcome this limitation but are not practicable in clinical situations. To exploit the possibilities of plasma decontamination in endodontics, a device has been developed with a flexible nozzle with a submillimeter diameter so it can be inserted down to the apex. The plasma therefore comes directly in contact with the bacteria. The present study aimed to verify the disinfection efficacy of the plasma jet generated by this novel device against

various endodontics-relevant bacteria on agar plates, particularly *E. faecalis*, which is considered the problem germ in this field.[22]

II. EXPERIMENTAL SETUP

A. Device

The plasma is generated using a pulse DC power source. The pulses are created from a custom-made electrical circuit that converts power from the high-voltage, low-current DC power source (Heinzinger PNC 3500-50 UMP, Rosenheim, Germany) to short pulses of higher current amplitude (150 mA). A pulse generator of low voltage and low current (Tektronix AFG 2021, Beaverton, Oregon) drives the IGBT transistor of the custom-made electrical circuit, thus controlling the frequency (1 kHz) and the width of the pulses (20 µs). Plasma jet on-time is 5% of treatment time. This allows the temperature of the jet and the substrate to be maintained below 40°C. A schematic of the electrical circuit has been presented elsewhere.[23]

The gas used is a mixture of helium and 1% oxygen, with a total gas flow of 1 standard liter per minute (SLM). The plasma streams through a flexible nozzle with an outer diameter of 0.3 mm and an inner diameter of 0.25 mm.

B. Method

Bacteria were grown in a tryptic soy broth (TSB) medium until the stationary phase and corresponded to a concentration of 10^9 bacteria/mL. This concentration was confirmed by dilution series and colony-forming unit (CFU) counts. 200 µL of undiluted solution was spread on Petri dishes. Excess humidity was removed in a cabinet (20 min at 37°C) so that the bacteria adhered to the agar surface and were not blown aside by gas flow. The Petri dishes were then transferred to a refrigerator (8°C) and removed 15 minutes before treatment to reach the room temperature.

Treatment time was 3 min per point, which is considered practicable for the disinfection step during a typical root canal procedure. The treatment was carried out by an adapted 3D printer and took place in a closed volume (30 L) to ensure reproducibility of the results. The distance between the nozzle and the agar surface was continuously varied from 1 to 3 mm over the 3-min treatment. The Petri dishes were then placed in an incubator (37°C) until colonies formed from the individual bacteria.

The treatment was tested on Gram-positive *E. faecalis* (T9), *Streptococcus mutans* (DSM 20523), and *Staphylococcus aureus* (ATCC 25923) as well as Gram-negative *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Images of the treated areas on agar plates were obtained using a camera (AxioCam MRc5, Zeiss, Jena, Germany) mounted on a microscope (Axioskop 2 MAT, Zeiss, Jena, Germany). The images were evaluated using ImageJ v. 1.52q software (National Institutes of Health, Bethesda, Maryland). Emphasis was on the size of the inhibition zone in which

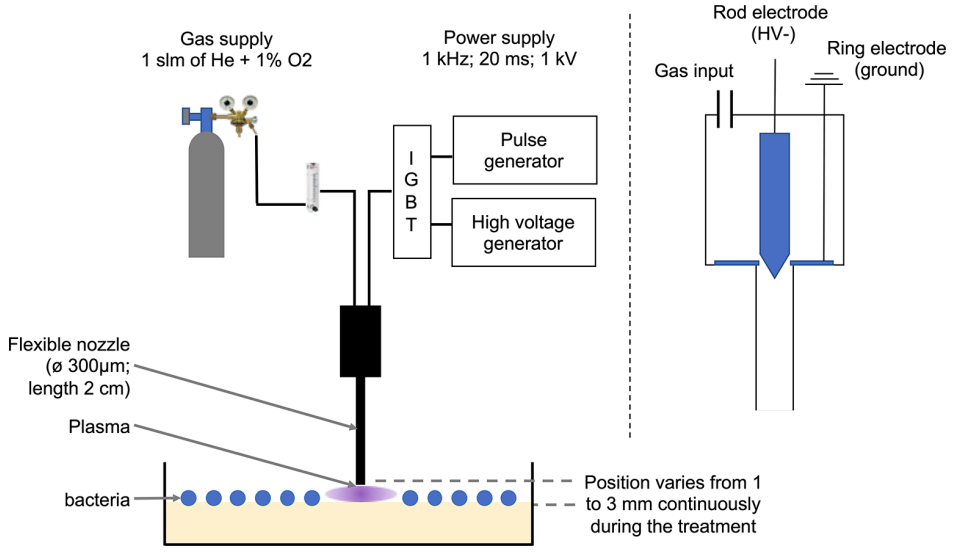

FIG. 1: (Left) schematic of the experimental setup. (Right) schematic of the jet interior.

a reduction in bacterial load was observed and on the number of CFUs in that area. A schematic of the experimental setup is shown in Fig. 1.

III. RESULTS

A. Transition Zone

Figure 2 shows three zones on a Petri dish after treatment with the plasma jet. The disinfecting effect of the plasma jet extended beyond the treated area into a transition area that developed between the treated area and the bacterial lawn.

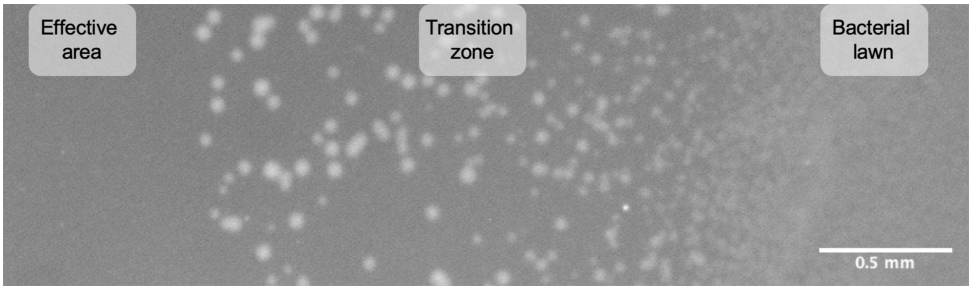

FIG. 2: Transient spark disinfection extending beyond the effective area to build a transition zone. (Right) *E. faecalis* bacterial lawn.

B. Microscopic Imaging

Microscope images of treated areas are shown in Fig. 3. The agar was carved by the gas stream expelled from the nozzle, creating a white dot and a slightly darker region. The first examination showed that the agar surface area directly under the nozzle (0.05 mm^2) was always completely free of bacteria, even with the undiluted bacterial solution (10^9 bacteria/mL). This indicated that the jet's region of action was much wider than the cross-sectional surface area of the nozzle.

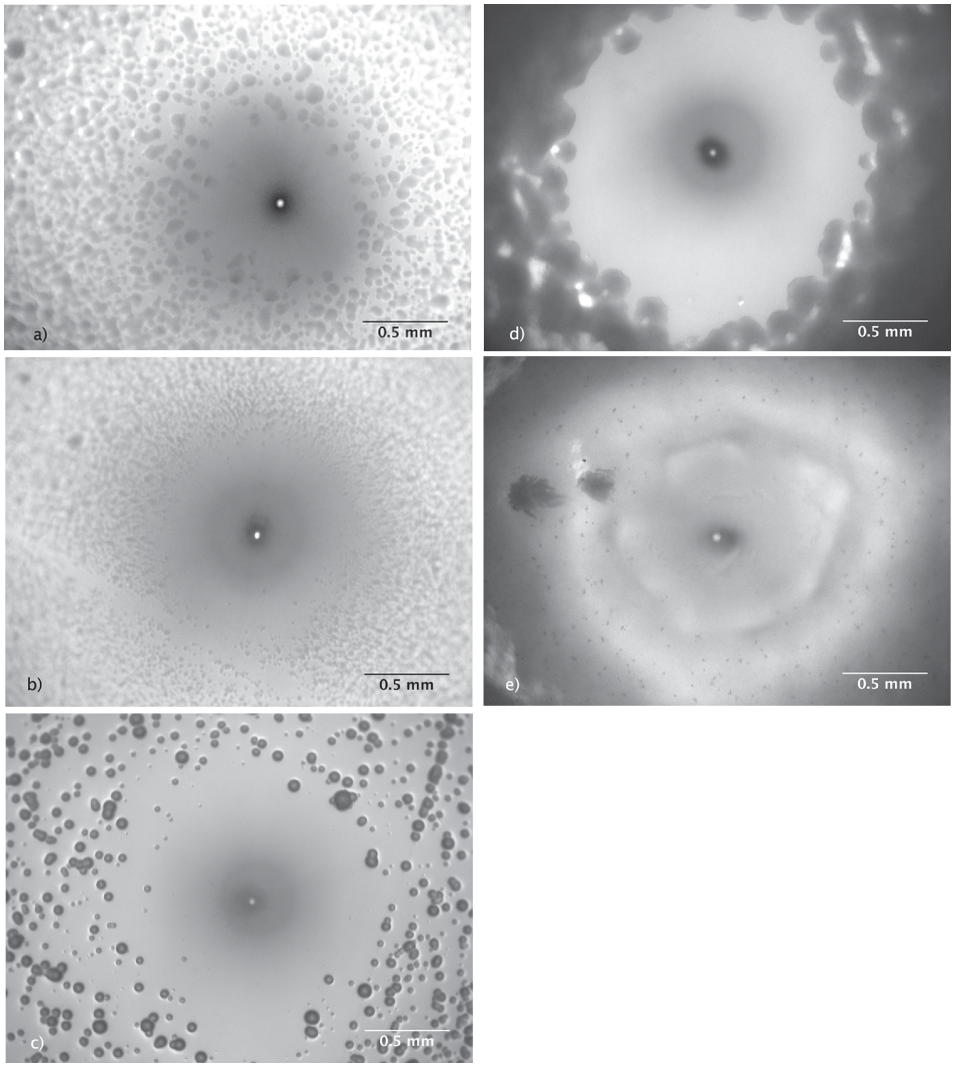

FIG. 3: Treated area on the agar. The impression of the gas stream is in the center of each image: around the center, no detectable bacteria after incubation; on the edge, transition to the bacterial lawn. a) *E. faecalis*, b) *S. aureus*, c) *S. mutans*, d) *E. coli*, e) *P. aeruginosa*.

C. Disinfection Efficacy

For each treated area, the surface area of the zone with no bacteria was evaluated. Figure 4 shows the distribution function of the bacteria-free surface area for *E. faecalis*. The median value of the area free of CFUs was 0.25 mm^2—that is, about five times the size of the nozzle surface area. The latter, represented by the dotted line in the graph, complied with an average apical enlargement size for standard root canals.

In order to compare the disinfection efficacy of the plasma jet, the bacterium-free area was determined in all experiments. Figure 5 shows the result in the form of a

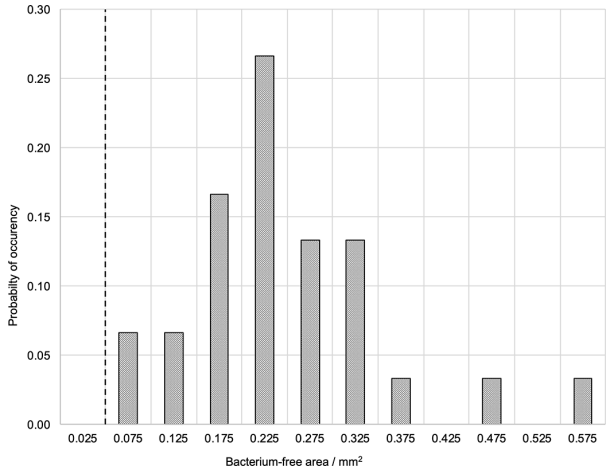

FIG. 4: Distribution function of the surface area with no *E. faecalis* CFUs after 3-min treatment. Dotted line shows the plasma jet surface area (i.e., the endodontics-relevant target surface area).

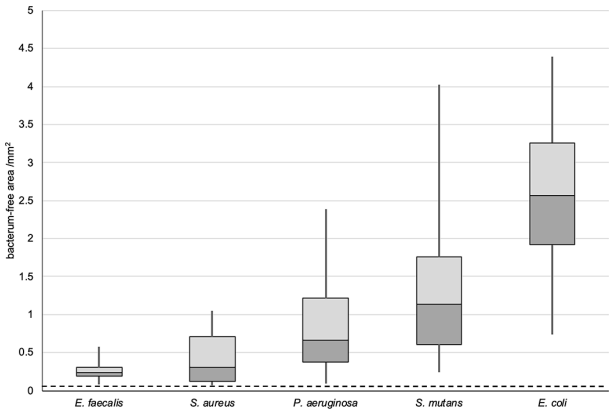

FIG. 5: Boxplot of bacterium-free surface area. *E. faecalis* (n = 30); *S. aureus* (n = 21); *P. aeruginosa* (n = 44); *S. mutans* (n = 48); *E. coli* (n = 46).

boxplot. indicating the median value, the IQR (boxes), and the minimum and maximum values (whiskers). In the figure, the disinfecting efficacy differs from germ to germ, confirming that *E. faecalis* is the most resistant bacteria and *E. coli* is the least resistant. The dotted line indicates the bacterium-free surface area, which is always larger than the target area in root canal treatment.

IV. CONCLUSIONS

This work investigated the disinfection effect of transient spark disinfection on various endodontics-relevant bacteria in Petri dishes, using undiluted solutions of 10^9 bacteria/mL. The median size of the bacterium-free area in the case of *E. faecalis* was 0.25 mm^2, which corresponded to five times the area of the plasma nozzle and matched the target surface area in root canal treatment. This value was even larger for the other investigated bacteria. *E. coli*, with the largest median bacterium-free surface area (2.5 mm^2), was the easiest bacterium to kill.

Ongoing work includes investigations specific to endodontics: the effect of transient spark disinfection on biofilms, through dentin, and in root canals. Further investigations are being designed to compare the efficacy of this plasma jet with that of other devices according to Mann et al.'s recommendations.[24]

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