The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 2359-2362

Assessment of Plasma Jet Therapy of Tooth Root Canal Infected with Escherichia Coli and Enterococcus Faecalis Biofilm In Vitro

Tamara A. Hameed[1], Hammad R. Humud[1], Layla F. Ali[2]
[1]Department of Physics and [2]Department of Biology, College of Science, University of Baghdad, Iraq
Corresponding author: Tamara A. Hameed
Email: tamara.aboud1104a@sc.uobaghdad.edu.iq **Mobile:** +964772671768

ABSTRACT

Background: Common and persistent isolate ina the teeth following failed therapy of the root canal is the gram-positive facultative bacterium *Enterococcus faecalis* and *Escherichia coli*, which develop biofilm through a complicated process that results in the formation of a biofilm. *Enterococcus faecalis* and *Escherichia coli* are significant factors that cause chronic periradicular lesions after root canal therapy.

Aim: This study aimed to treat the root canal tooth infected with Escherichia coli and Enterococcus faecalis

Methods: In this study biofilm formation was done for *Escherichia coli* in growth phase cultured in a brain heart broth *Enterococcus faecalis* and *Escherichia coli* cultured in Luria-Bertani (LB) infusion medium for 18 hrs. Then we studied the effect of plasma jet that works with argon gas, and it is generated by a power supply that operates at alternating high voltages in the form of a sinusoidal wave with peak-to-peak value of about 12 kV at a frequency of 30 KHz and its power is about 200 watts.

Results: *Enterococcus faecalis* biofilms were treated for 0.5, 1, 1.5, 2, 2.5 minute with a constant gas flow rate 2.5 L/min. The killing rate decreased from (0.979 to 0.361). In the *Enterococcus faecalis* biofilms was treated for a longer period of time (5, 10, 15, 20, 25, 30) min, as the killing rates decreased from (0.739 to 0.179).

Conclusions: The result of this study indicates that plasma jet is useful in disinfecting root canal tooth and reducing the formation of biofilm.

Keywords: Plasma jet, *Enterococcus faecalis*, *Escherichia coli*, Root canal tooth, Biofilm.

1. INTRODUCTION

Endodontic therapy's ultimate objective is to clear the root canal system of the microorganisms and promote apical periodontitis healing [(1,2)]. Routine intracanal procedures, such as using mechanical instruments, intracanal irrigants, and medications with antibacterial properties may be used to remove the majority of pathogenic bacteria and their primary necrotic pulp debris substrate. However, both single- and multiple-visit root canal procedures have commonly been followed by persistent periradicular infection [(3,4)]. The primary factor contributing to failure has been identified as the persistence of the bacteria in apical area of root-filled tooth [(5)].

A common and consistent isolate in the teeth following failed therapy of the root canal is the gram-positive facultative bacterium Enterococcus faecalis [(6)]. Escherichia coli develops its biofilm through a complicated process that results in the formation of a biofilm, which is crucial in the development of a number of diseases caused by bacterial attachment and resistance to a broad range of antibiotics. E. faecalis and E. coli are significant factors in causing chronic periradicular lesions after the therapy of the root canal. Its incidence in the teeth that had filled roots with periradicular lesions ranged from 24 to 77 percent, according to reports [(2)].

According to certain theories, microbial development as biofilm could help microorganisms survive challenging growth environments, including those seen in post-endodontic environment of the root canal. Bacteria form a sessile microbial community known as a biofilm when they are encased in an extracellular polymeric matrix that they self-create. Because of the changed bacterial phenotype and biofilm matrix, the biofilm has a lower vulnerability to drugs and human immune responses. Bacterial biofilms are therefore thought to be frequent cause of many oral illnesses, which include the pulpitis, dental caries, damage of the root canal tooth, and periradicular lesions [(7)]. The latest advancements in the non-thermal, atmospheric-pressure plasmas have made it possible to employ the plasma to eliminate the bacteria linked to diseased root canals after drilling and cleaning teeth [(8)].

The 4th state of the matter, plasma, represents a quasi-neutral mixture of neutral and charged species. Whereas bulk gas remains close to the temperature of the room, a non-thermal, atmospheric pressure plasma facilitates production of the reactive chemical species and their interactions with items being treated. These characteristics made those plasmas very desirable in wide range of environmental and bio-medical applications, such as low-heat surface modification of polymers, food processing and medical instruments' sterilization [(9)].

Plasma plume for root canal disinfection was created using a temperature of the room plasma dentistry probe. The plasma plume can introduce reactive plasma species (such as charged species and reactive oxygen species (ROS)) that are able to penetrate anywhere in root canal, which includes via dentinal tubules, and clean surfaces by the bactericidal processes[(3)].

Received: 19/07/2022
Accepted: 24/09/2022

The antibacterial effectiveness of plasma jet against E. faecalis and E. coli biofilms has been evaluated in this in vitro investigation. In the present work, we have researched effectiveness of the disinfection of an innovative cold plasma jet for the in vitro inactivation of the E coli and E. faecalis biofilms in the root canals.

2. EXPERIMENTAL SETUP

2.1. Plasma Jet System Setup

An image of non-thermal atmospheric pressure plasma jet (USA) at work is depicted in Fig. 1. It includes a Pyrex glass tube and aluminum foil, 10 mm wide, placed around the Pyrex glass tube 20 mm away from its end. The power supply of high voltage is connected to aluminum foil, this power supply produces high voltage with a sinusoidal shape of 12 kV, a peak-to-peak, and a frequency of 20.0kHz. We worked with compressed argon gas at a 2.50ℓ/min flow rate. A plasma jet with a visible length of around ≤1.5 cm was produced under such operating circumstances. Utilizing a thermometer, it was found that the gas temperature was approximately 38°C, just 1 cm from the nozzle.

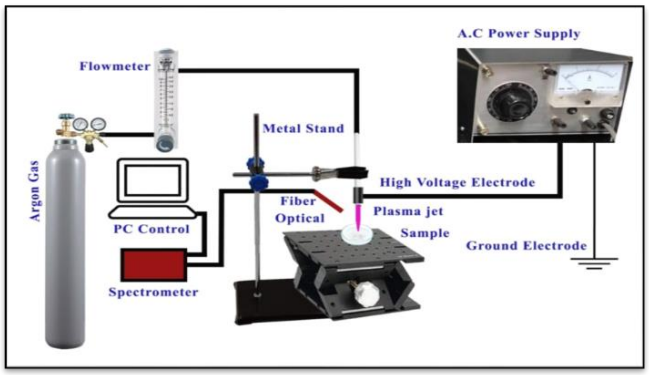

Figure 1: Plasma jet system at working

2.2 Bacterial Growth and Root canal samples

Before the experiment, single-rooted extracted, the unharmed permanent teeth were chosen and preserved at a temperature of 4 Celsius in a 0.1% thymol solution. Using the step-back approach, root canals were prepared using Ni-Ti hand files (Mani Inc., Japan) up to #40, and debris was removed by irrigating whenever the size of the file was changed. For injecting bacteria inside the root canal, every one of the apical foramens was sealed with composite resin (Clearfill AP-X, Kuraray Dental, Japan). Following this process, the root canals created a cone-shaped cavity (with a volume of about 10μL) with a narrow bottom that is sealed. All of the samples were sterilized in an autoclave prior to any additional treatments. In order to get *Escherichia coli* into the growth phase, it was cultured in a brain heart and Enterococcus faecalis was cultured in Luria-Bertani (LB) infusion medium for 18 hrs. Root canals received 10 μL of a fresh, diluted suspension that contained 10^7 CFU per mL. Because it is comparable to the actual clinic situation, such a concentration of bacteria was selected. Figure 2. shows plasma jet treatment on extracted single root canal of the human tooth.

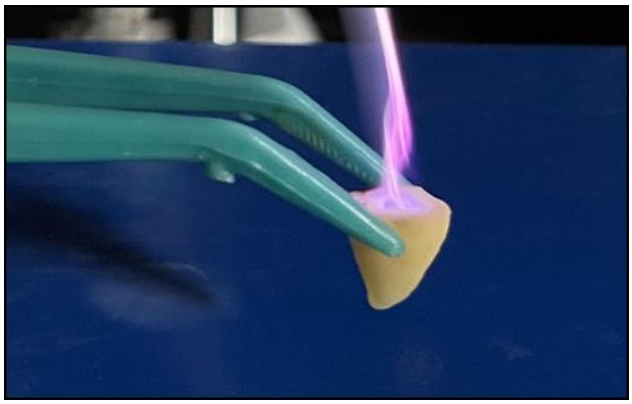

Figure 2. Picture of plasma jet treatment on an extracted single-root human tooth

2.3 Bio film formation

The general procedure: Identified isolates were cultured overnight (about 7 week) at a temperature of 37ºC in brain heart infusion broth. Each isolate was diluted with tryptic soy broth (TSB), which contains 1 percent glucose, and well mixed using a pipette. A suspension of a bacterial isolate was brought up to the McFarland No. (0.5) turbidity criterion. Three of the sterile, 96-well polystyrene micro plates three wells each received a volume (200 ml) of each an isolate culture. The plates were covered with lids and incubated in an aerobic manner for 24 hrs at 37ºC.

After the period of incubation, the planktonic cells were rinsed twice with deionized water to remove unattached bacteria. The surplus water was then wiped off the plate using filter paper, dried, and fixed at 65°C for 1 hour after being rinsed twice with deionized water to remove excess water by tapping the plate on filter paper. 200 ml of 100% methanol were used to fix the adhering bacterial cells in each well for 20 min at room temperature. The adherent cells were stained by the addition of 200 ml of 0.10 percent crystal violet to every one of the wells and letting it sit for 15 minutes. Repeated washing with distilled water (2–3 washes) after the staining reaction had finished helped to remove the extra stain. The plates were dried for around 20 minutes at room temperature to make sure they were completely dry. Then 200 ml of glacial acetic acid at a concentration of 33% was added for 10 minutes. The absorption of wells containing TSB free of microorganisms was employed as a negative control in the triplicated experiment.

The amount of crystal violet produced in each well was directly quantified spectrophotometrically by measuring the OD490 using a micro plate reader. To simplify and calculate the data. The above steps were

repeated for each of the transactions of each treatment with plasma jet.

2.4 Treatment of plasma jet on bacterial biofilm

The above steps were repeated for each of the transactions of each treatment with plasma jet.

2.5 Ethical approval:

The study was approved by the laboratory of the Environmental Center at the University of Baghdad. All participants agreed to participate in the study after signing an informed written permission form. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

2.6 Statistical Analyses

Statistical Analysis System- SAS (2012) application has been employed to determine how various factors affected the study parameters. The present work's means were compared using the LSD test (ANOVA). All data were represented as mean ± standard deviation. Statistical significance was considered as P<0.01

3. RESULT

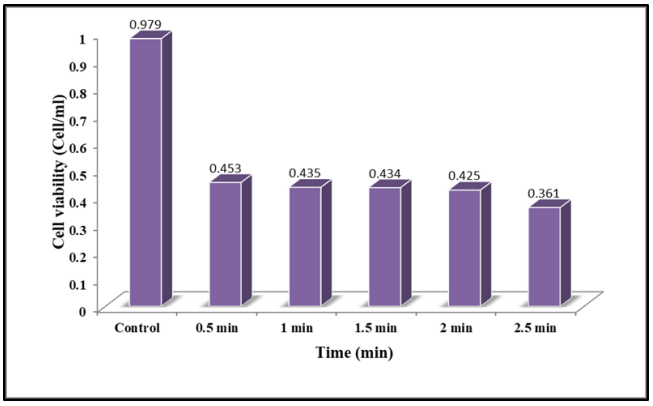

Figure 3. The effects of Argon plasma treatments of Escherichia coli biofilms in root canal. Escherichia coli biofilms were treated for 0.5, 1, 1.5, 2, 2.5, minutes

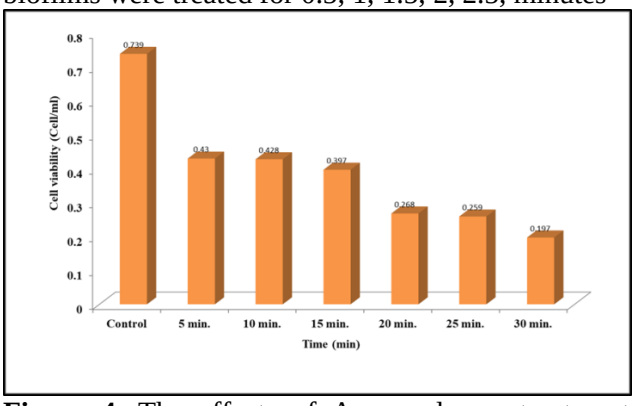

Figure 4. The effects of Argon plasma treatments of Enterococcus faecalis biofilms in root canals. Enterococcus faecalis biofilms were treated for 5, 10, 15, 20, 25, 30 minutes.

The figure (3) and (4) show the effect of plasma jet treatment for Escherichia coli and Enterococcus faecalis biofilms in the root canals of the teeth, which were the treated at different times. Where we notice from the figure a reduction in number of the CFU units after the plasma treatments for different time, and the degree of inactivation increases with the increase in the time of exposure to plasma, and through Figure 3 and Figure 4 we note that Enterococcus faecalis were exposed for longer periods 5, 10, 15, 20, 25, 30 minutes while Escherichia coli for shorter periods 0.5, 1, 1.5, 2, 2.5 minutes.

4. DISCUSSION

Enterococcus faecalis has been known to have high antibacterial resistances and has thus been detected frequently in apical lesions that are persistent. Elimination of residual micro-organisms within biofilm in channel system the complex root is an important determination. The effective inactivation of biofilm in the root canals of the tooth is due to charged particles, excited species, and ultra-violet rays that are generated in non-thermal atmosphere of plasma jet. Potential mechanisms that play a role in inactivation are direct destructions by the ultra-violet rays and the energetic charged particles; erosion micro-organisms by the atomic oxygen or other radicals that emanate from plasma jet. In addition to the rupture of the membrane by the bacteria by strong Coulomb force that results from the accumulation and inactivation of the charge by the long-lived reactive species that have been dissolved in the water on tooth root canal surface that later creates reactive radicals like O_2 and OH. Biofilms have been associated to big challenges to the control infection, even by broad range antibiotic medications. The main objective in the health-care is finding a new strategy for the limitation of biofilm formation by the bacteria that are hard to get rid of through the use of the conventional approaches, particularly using antibiotics. In the case where the scientific research has the ability to find such approach, which will lead to the occurrence of an important advancement in the reduction of pathogenic bacteria spread [(10)]. Earlier research had shown that bacteria exposure to sub-lethal dosage of the plasma results in the removal of bacteria activity in vitro [(11)]. Which is why, effects of the non-thermal plasma against the biofilm proposed being advisable in the treatment of the formation of the biofilm by a number of the bacterial species in vitro. The effects of the non-thermal plasma upon a variety of the physiological bacterial cell activities have been reported by earlier research [(12)].

From the present research, it may be concluded that non-thermal plasma may result in the reduction of biofilm and that could result in opening a broad range of the applications which could result in solving the biggest issue in bio-science field.

5.CONCLUSION

In this paper, room temperature plasma jet had shown anti-microbial effects on *E. coli* and *E. faecalis* biofilms. Besides shown biofilm-removal effect, cold plasma could be safer when compared to traditional medicament irrigations as improved oxidation that has been provided by the reactive plasma species is more localized. Nevertheless, more researches are required for the assessment of cold plasma-based technology effectiveness and feasibility for the disinfection of the root canal.

Declaration of conflict of interest: There was no disclosure of any possible conflicts of interest related to the research.

Funding: no funding

6. REFERENCES

1. **Abbas K, Hussein U (2017):** The study of electrical description for non-thermal plasma needle system. Iraqi Journal of Science, 58(3):1447-1453.
2. **Stuart H, Schwartz A, Beeson J *et al.* (2006):** Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. *Journal of endodontics*, *32*(2): 93-98.
3. **Shahwany W, Tawfeeq K, Hamed E (2016):** Antibacterial and anti-biofilm activity of three phenolic plant extracts and silver nanoparticles on Staphylococcus aureus and Klebsiella pneumoniae. *J Biomed Biotechnol.*, *4*:12-18.
4. **Vivacqua-Gomes N, Gurgel-Filho D, Gomes A *et al.* (2005):** Recovery of Enterococcus faecalis after single-or multiple-visit root canal treatments carried out in infected teeth ex vivo. *International Endodontic Journal*, *38*(10): 697-704.
5. **Al-wusaybie M, Al-Ramil M, Al-Wosaibi M *et al.* (2018):** Prevalence of impacted teeth and associated pathologies–A radiographic study, Al Ahsa, Saudi Arabia Population. The Egyptian Journal of Hospital Medicine, 70(12): 2130-2136.
6. **Sedgley M, Lennan L, Clewell B (2004):** Prevalence, phenotype and genotype of oral enterococci. *Oral Microbiology and Immunology*, *19*(2): 95-101.
7. **Estrela C, Sydney B, Figueiredo P *et al.* (2009):** Antibacterial efficacy of intracanal medicaments on bacterial biofilm: a critical review. *Journal of Applied Oral Science*, (82)17: 1-7.
8. **Jiang C, Chen T, Gorur A *et al.* (2009):** Nanosecond pulsed plasma dental probe. *Plasma Processes and Polymers*, *6*(8): 479-483.
9. **Lee N, Paek H, Ju T *et al.* (2006):** Sterilization of bacteria, yeast, and bacterial endospores by atmospheric-pressure cold plasma using helium and oxygen. *Journal of Microbiology*, *44*(3): 269-275.
10. **Gilbert P, Allison G, McBain J (2002):** Biofilms in vitro and in vivo: Do singular mechanisms imply cross-resistance. *J Appl Microbiol.*, 92(10): 98S-110S.
11. **Cooper M, Fridman G, Fridman A *et al.* (2010):** Biological responses of Bacillus stratosphericus to floating electrode-dielectric barrier discharge plasma treatment. *J Appl Microbiol.*, 109: 2039-2048.
12. **Ghafil A (2018):** Assessment the effect of non-thermal plasma on Escherichia coli and Staphylococcus aureus biofilm formation in vitro. Iraqi Journal of Science, 59(1): 25-29.