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Comparison Study of Plasma-Activated Water and Plasma Jet Effects on *Escherichia coli* Bacteria for the Disinfection of Tooth Root Canal

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Abstract
This study compares the effects of plasma jet and plasma-activated water on teeth root canals contaminated with *Escherichia coli* bacteria. A plasma jet system was developed for biological purposes that operate at atmospheric pressure. The plasma jet works with argon gas and is generated by a power supply, which supplies a sinusoidal alternating voltage of 12 kV of 20 kHz frequency. The system was optically diagnosed, as it was found that the peaks of the nitrogen spectrum were obtained at the wavelength (300- 450) nm with the appearance of hydroxide peaks at 380 nm. Extracted teeth with one root canal were used, which were contaminated with bacteria and divided into two groups to be treated with a plasma jet and plasma-activated water. The first group was treated with a plasma jet at a gas flow of 2.5 L/min with a change of treatment time from 0.5 to 3 minutes. The number of killed bacteria colonies increased when the exposure time increased (2.7×10^{8} CFU per mL - 0), and the total killing of bacteria was obtained at 3 minutes treatment time. While the second group was treated with plasma-activated water; the water used to kill the bacteria was treated with plasma for one hour at a gas flow rate of 1 L/min. The measured concentrations of (NO_2, NO_3, and H_2O_2) of this water were (10, 100 and 200) ppm. The teeth were treated with plasma-activated water for different times that ranged between (5- 35) minutes. Different bacterial killing rates were obtained at these times, and the total bacterial killing rate was obtained for 35 minutes of treatment time. The obtained results found that the response of bacteria to treatment with plasma jet is better than treatment with plasma-activated water.

Keywords: Plasma jet, Activated water, Root canal tooth, Reactive species, *Escherichia coli*

دراسة مقارنة تأثيرات الماء المنشط بالبلازما ونافث البلازما على بكتيريا الاشريكية القولونية لتطهير قناة جذر السن

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الخلاصة:
يدرس هذا البحث تأثيرنافث البلازما والماء المنشط بالبلازما على قنوات جذر السن الملوثة ببكتيريا الإشريكية القولونية. استخدام أنظمة البلازما النفاثة المطورة للأغراض البيولوجية التي تعمل تحت الضغط الجوي

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. البلازما النفث تعمل بغاز الأرجون ، وتتولد البلازما عن طريق مصدر تيار متناوب يجهز جهد متناوب عالي الجهد 12 كيلو فولت عند 20 كيلو هرتز. تم تشخيص النظام طيفياً حيث وجد أن قمم طيف النيتروجين تم الحصول عليها عند الطول الموجي (300−450) نانومتر مع ظهور قمم الهيدروكسيد عند 380 نانومتر. تم استخدام أسنان مقلوعة ذات قناة جذر واحدة ملوثة بالبكتيريا وقسمت إلى مجموعتين لمعالجتها بالبلازما النفث والماء المنشط بالبلازما. عولجت المجموعة الأولى بنفث بلازما بتدفق غاز 2.5 لتر / دقيقة وتم التغيير في وقت المعالجة من 0.5 إلى 3 دقائق. حيث زادت عدد المستعمرات البكتيريا المقتولة عندما ازداد زمن التعريض (2.7×10^8−0) وحدة تشكيل مستعمرة لكل مليلتر ، وتم الحصول على معدل قتل البكتيريا في 3 دقائق. بينما عولجت المجموعة الثانية بماء منشط بالبلازما حيث تمت معاملة الماء المستخدم لقتل البكتيريا بالبلازما لمدة ساعة بمعدل تدفق غاز 1 لتر / دقيقة والتراكيز المقاسة (NO_3,H_2O_2, NO_2) في هذه المياه (10 و 100 و 200) جزء في المليون. عولجت الأسنان بالماء المنشط بالبلازما في أوقات مختلفة تراوحت بين (5 − 35) دقيقة ، حيث تم الحصول على معدلات قتل مختلفة في هذه الأوقات ، ومعدلات قتل البكتيريا الكلية عند 35 دقيقة. أظهرت النتائج أن استجابة البكتيريا للعلاج البلازما جت أفضل من العلاج بالماء النشط بالبلازما.

1. Introduction

Plasma consists of charged particles, electrons, photons, UV, and free radicals. The components of plasma, such as singlet oxygen and free radicals, have antibacterial properties [1]. These species can inactivate cells and cause cell lysis by oxidation resulting in decontamination and sterilization [2]. Plasma sterilization has widely been used in dentistry for tooth bleaching [3], removal of plaque [4], sterilization of dental equipment and implants [4], and disinfection of root canals. So, plasma can be used for disinfection.

Plasma-Activated Water (PAW) is the other method for disinfection that has been successful in endodontic disinfection. In this method, The energetic particles from the plasma phase are trapped in the solution during PAW production, causing a chain of events to be initiated at the gas-liquid interface, leading to the dissolving of a variety of reactive primary and secondary species in water. Reactive Oxygen Species (ROS) are produced primarily due to the presence of oxygen in the environment, leading to the production of toxic oxygen species, such as atomic oxygen and free radicals. ROS can kill a wide range of microbes in tooth root canals [5–8]. Plasma jet has shown efficacy against oral microorganisms, in addition to anti-inflammatory properties, with possible application in cardiology, endodontics, periodontics, and oral oncology [9]. Besides, plasma-activated water (PAW) has also demonstrated potential application in dentistry [10].

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 [7]. Traditional treatments such as mechanical debridement, chemical irrigation, and ultrasound can significantly reduce the population of bacteria inside the infected root canal. However, the elimination of all bacteria from the root canal is hard. This is attributed to the complex morphology of the root canal [2]. Multiple studies have shown that E.coli is a commonly isolated bacteria associated with persistent periapical lesions. E.coli is a gram-negative facultative bacterium that can live in dentinal tubules for long periods under nutritional deprivation. The biofilm form of bacteria shows more resistance to antibacterial and antibodies than planktonic bacteria. When E.coli grows in the root canal system, it becomes more resistant, although it can be easily destroyed in an open environment [4].

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 found to be efficient against young and mature E. coli. Nishime et al. [11] evaluated the sterilizing efficacy of a non-thermal plasma jet toward gram-positive and gram-negative bacteria and demonstrated the sterilizing efficacy. Maisch et al. [12] evaluated the antimicrobial effects and the mechanism of cold atmospheric plasma treatment of E. coli bacteria. They showed that the bacteria inactivation increased with a longer treatment time. Vlad et al. [13] investigated the bacterial inhibition effects of plasma-activated water on *Staphylococcus aureus*, which proved to be efficient for bacterial inhibition.

Although numerous studies have investigated the antibacterial effects of plasma and PAW against E.coli, no study has compared the effects of the two techniques. Comparing different methods can help find an effective and efficient way to solve 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, a plasma jet system was built to treat tooth root canal bacteria by exposing it to plasma-activated water for different exposure times and comparing the results to those of direct exposure to a plasma jet.

2. Experimental Setup

2.1 Plasma Jet System Setup

A schematic diagram of the setup of a non-thermal atmospheric pressure plasma jet is shown in Figure 1. It includes a pyrex glass tube (with an inner diameter of 5 mm) wrapped up with a 10 mm wide aluminium foil 20mm away from its end. A high-voltage power supply (producing alternating sinusoidal high voltage of 30 kV peak-to-peak and frequency of 20 kHz) was connected to an electrode made of the aluminium wrapped at the end of the glass tube at a distance of one centimeter from its end. Compressed argon gas at a flow rate of roughly 2.5 L /min was used in this system. A plasma jet with a visible length of around ≤1.5 cm was produced under such operating conditions. With a thermometer, the gas temperature, 1 cm away from the nozzle, was measured to be approximately 34°C. The electron temperature (T_e = 0.750 eV) and electron density ($n_e = 5.405\times10^{17}$ cm^{-3}) were calculated at an argon flow rate of 2.5 L/min. As the distance between the sample and the plasma torch increases, the measured temperature of the gas gradually decreases.

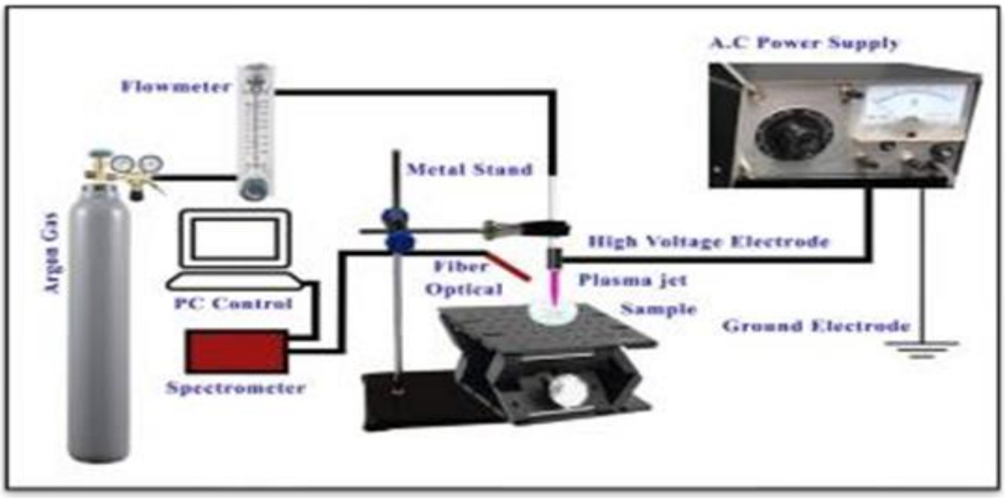

Figure 1: Plasma jet system.

2.2 Root canal samples and Bacterial Growth

Before the experiment, single-rooted extracted, intact 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 each time the file size was changed. For injecting bacteria inside the root canal, each apical foramen was sealed with composite resin (Clearfill AP-X, Kuraray Dental, Japan). Following this process, the root canals were cone-shaped cavities (about 10µL volume) with a sealed narrow bottom. All the samples were sterilized in an autoclave before any additional treatments. To get *Escherichia coli* into the growth phase, it was cultured in a brain-heart infusion medium for 18 hours. Root canals received 10µL of a fresh, diluted suspension of *Escherichia coli* bacteria that contained 10^7 CFU per mL. Such a concentration of bacteria was selected because it is comparable to the actual clinic situation.

2.3 Plasma activated water

To prepare the PAW, ten milliliters of pure distilled water (whose temperature and acidity were measured) in a petri dish was exposed for one hour to the plasma jet. After completing the water treatment, the concentrations of H_2O_2, NO_3, and NO_2 were measured using test strips (Bartovation, USA), as shown in Table 1. The PH was measured with a pH meter (PH-009(1), China), and temperature was measured using a remotely IR thermometer.

Table 1: The concentrations of H_2O_2, NO_3 , and NO_2

Water Treatment	**Time (min.)**	**60**
	Flow (*l/min.*)	0.5
Chemical compound	Concentration (ppm)	
NO2	10	
NO3	100	
H2O2	200	
PH	3	
Temp. Before	18 C^0	
Temp. After	34 C^0	

2.4 Treatment method of root canal tooth

There are two approaches for treating bacteria in teeth root canals: direct plasma and indirect plasma (plasma-activated water) methods. Both methods were employed in this work. In the first method, an Ar gas plasma jet was used. Time and gas flow rate were employed to guide the treatment. The gas flow rate was fixed at 2.5 L/min, and the contaminated teeth were exposed to the plasma jet for different periods (0.5, 1, 1.5, 2, 2.5, 3, and 6) minutes. In the second approach, water that had undergone plasma treatment for an hour at a stable gas flow rate of 1 L/min was used. The contaminated teeth were placed in the activated water for various time intervals (5, 10, 15, 20, 25, 30, and 350) minutes. After the exposure, 100 microliters of brain-heart infusion broth was added and the teeth were allowed to sit for 20 minutes. Then, 100 microliters of brain heart infusion broth were added and allowed to sit for 20 minutes. Then 100 microliters of bacteria were added to a petri dish, distributed with the use of a swap, and then implanted with Macconkey agar Himedia (India) for 18 hours, or until the bacteria have reached the growth phase and bacterial colony were counted. Figure 2. shows plasma jet treatment on an extracted single human tooth root canal.

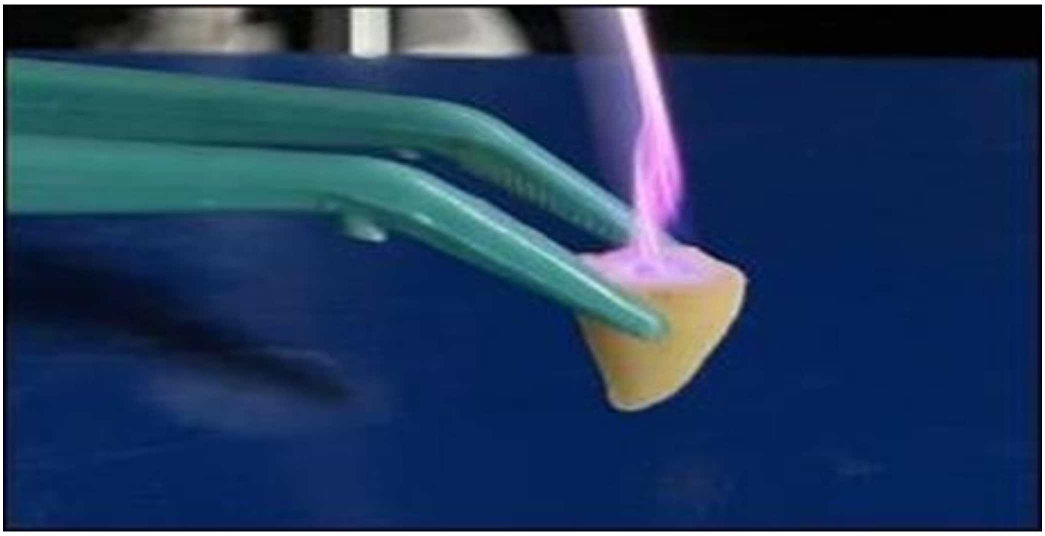

Figure 2: Plasma jet treatment on an extracted single-root human tooth

2.5 Statistical Analysis:

To determine how various factors affected the study parameters, Statistical Analysis System-SAS (2012) application was employed. The means in the present work were compared significantly using the LSD test (ANOVA).

3. Result and discussion

Figure 3 shows the nitrogen gas spectrum that was observed when diagnosing the plasma jet systems generated by Ar gas. Numerous nitrogen gas peaks (between 300 and 450 nm) are observed in the figure. The figure demonstrates the OH peak at a wavelength of 308 nm, which was also confirmed by the test strips, as shown in Table (1). The highest peak in the spectrum appeared at 357.69 nm, with the appearance of some lower peaks on its sides. This results in the generation of RONs compounds that aid in the disinfection of the tooth's root canal. The first method for disinfecting the tooth root canal was using the plasma jet. The gas flow rate was fixed at 2.5 L/min, and the teeth were exposed to the plasma jet for different periods (0.5, 1, 1.5, 2, 2.5, 3, and 6) minutes. Figure 4 represents the relationship between the rate of killing *E.coli* bacteria and the exposure time to the tooth root canal. The number of *E.coli* colonies before plasma exposure was 45×10^8 CFU/ml. The proportion of killing *E.coli* bacteria at the time (0.5-6) min was (2.7×10^8, 2.4×10^8, 1.5×10^8, 1.2×10^8, 0.883×10^8, 0)CFU/ml, respectively. It was noted that increasing the time of plasma exposure is an important factorin increasing the rate of killing. The bacteria were highly affected, where a 100% killing was obtained within three minutes only. No colonies were observed for exposure of more than 6 minutes, meaning the disinfection of the tooth root canal was achieved. The reason for the colonies killing is due to the generation of reactive species. It is also known that the oxygen produced from the interaction of plasma with air plays a vital role in cleansing the affected root canals by disrupting the cells and causing their decomposition by oxidative stress. In this study, a unipolar plasma jet was used on *E.coli* in teeth root canals, where the plasma temperature was 34^{o}C, so it has no harmful effects on living membranes. The results showed that plasma treatment effectively reduces the CFU inthe affected root canals; increasing the treatment time improved the degree of bacterial purification, as the free radicals generated by the plasma diffuse through the strong physical and chemical membrane of the bacteria quickly and interact directly with subcellular organelles and biomolecules. The plasma and reactive species' atoms and ions can interact directly with the bacteria. The accumulating charges on the cell membrane cause the membrane to rupture due to the Coulomb force. Some reactive species live for a long time, and decomposition occurs when interacting with the water on the tooth's surface, producing effective oxygen compounds. Those reactive species interact with the bacteria. The reactive species, OH, can be transported into the cytoplasm of the bacteria through a series of mechanisms. This result is consistent with that of Wang et al. [14]. Bacterial inactivation using plasma jets can occur through two different mechanisms: physical and chemical. Heat, ultraviolet radiation, and charged particles are examples of physical factors, while active species are examples of chemical agents. On the outside of the cell membrane, charged particles might accumulate due to plasma jet [3]. These charges combine to create an electrical force that can break the tensile force of the cell membrane and cause it to burst. Because of the rapid recombination of electrons and ions, when the plasma indirectly displays bacteria, the concentration of charged particles decreases [15]. Increasing the plasma exposure time of bacteria leads to an increase in the accumulation of charges, which results in an increase in the rate of killing of bacteria. From this, it becomes clear that the exposure time has a very important effect on the increase in the rate of killing (from 2.7×10^8 CFU per mL to zero). Since all bacteria samples in this investigation were indirectly exposed to the plasma column. As a result, the inhibition effect of ultraviolet radiation on microorganisms in this range is largely related to DNA [4].

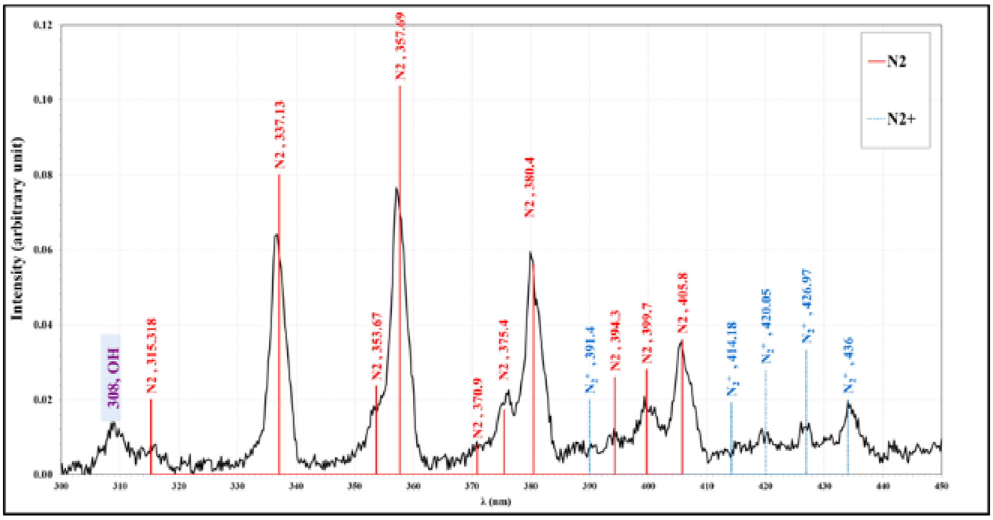

Figure 3: The emission spectra from (300-450) nm of N_2.

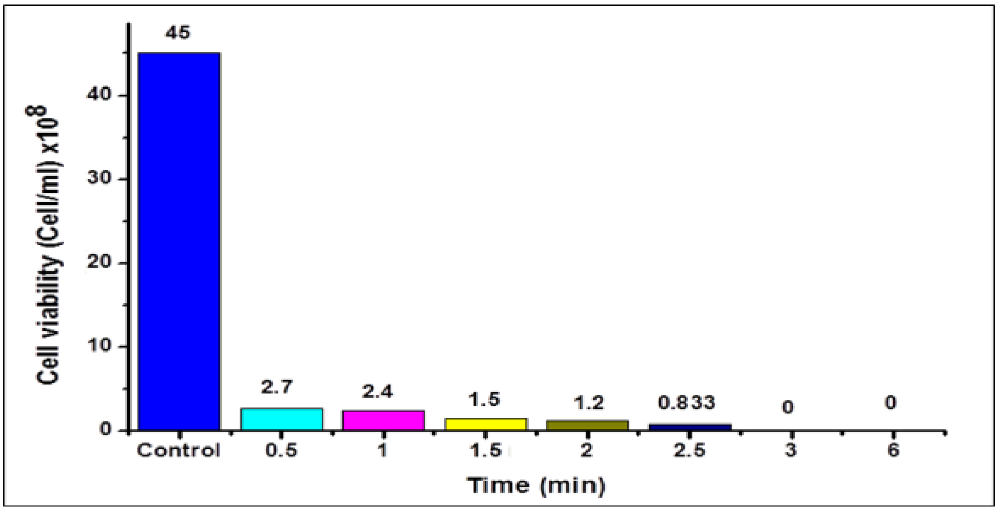

Figure 4: (a) Histogram showin the effect of plasma jet with different exposure times on the cell viability of *Escherichia coli*. All data have been represented as mean ± standard deviation LSD test (ANOVA). Statistical significance has been considered as $P<0.010$.

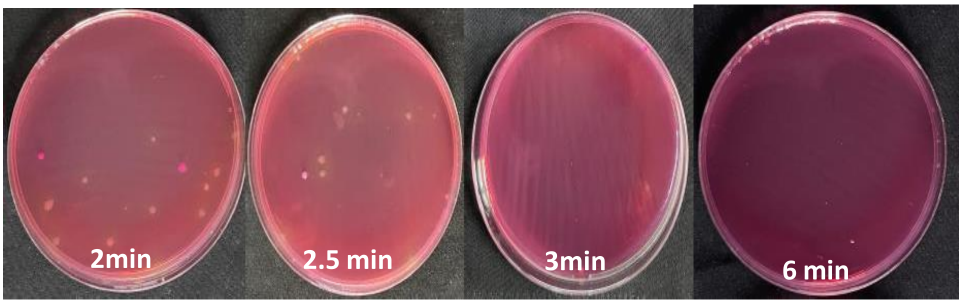

Figure 4: (b)*Escherichia coli bacteria* inactivation for different exposure times.

The second method for disinfecting the tooth root canal is using plasma-activated water with a time change from (5-35) min. Figure (5-a and 5-b) shows the relationship between the percentage of bacteria killing in the tooth root canal using plasma-activated water, where the teeth were treated with plasma-activated water for different times (5, 10, 20, 25, 30, 35) min, and the concentrations of RONs were as shown in Table (1). It was noted, from the results, that the effectiveness of PAW depends on the treatment time. The plasma treatment for 5 minutes is the least effective. When exposing water to plasma, the pH drops from 7 to 3. The pH alone does not cause disruption, but it plays an indispensable role in influencing the bacteria, as the compounds NO_2 and NO_3 cause water acidity. Water acidity plays an indispensable role in inhibiting the growth of bacteria. Also, the physical and chemical properties of plasma-activated water, including conductivity, pH, and the presence of reactive species H_2O_2, NO_2, and NO_3, helped to understand the inactivation mechanism better. During the generation of PAW, charged particles are absorbed in the gas phase and interact with water molecules, resulting in long-lived reactive species. It was also observed that the pH of the plasma-activated water decreased [16].

Comparing the results of the two methods, it was noted that E.coli bacteria showed more response when directly exposed to a plasma jet, where100% kill took place after 6 minutes of exposure to the plasma jet; however, it took more than half an hour when treating the teeth with plasma-activated water due to the protective effects of negative bacteria against the confirmed environment of PAW since activated water does not contain short-lived compounds, unlike direct exposure, which contains both short-lived and long-lived types. On the other hand, there are advantages to using plasma-activated water, it cancels the use of the plasma jet system, and there is no high temperature invoved that affects the teeth.

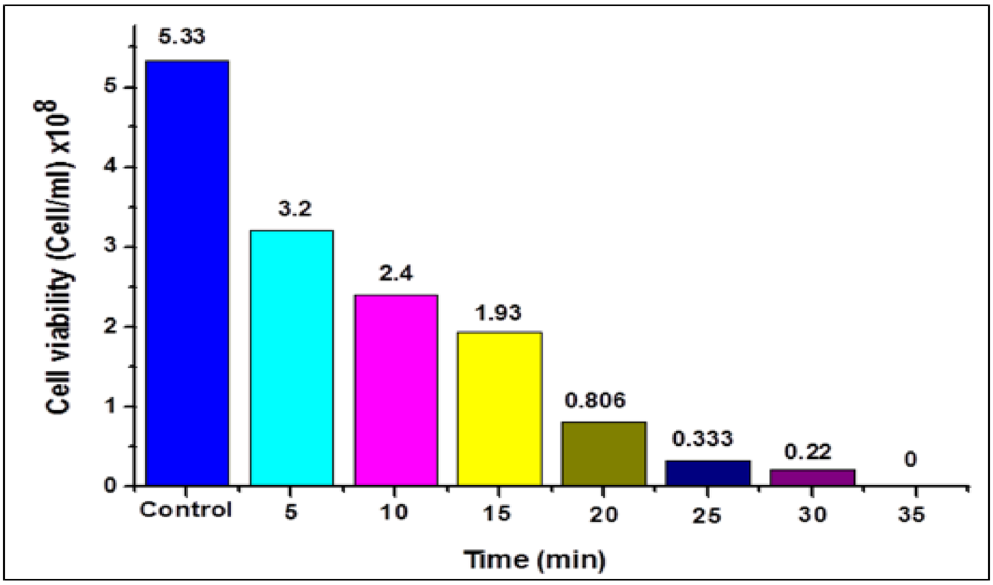

Figure 5: (a) Histogram shoeing the effect of plasma - activated water with different times on the cell viability of *Escherichia coli*. All data have been represented as mean ± standard deviation LSD test (ANOVA). Statistica significance has been considered as P<0.01

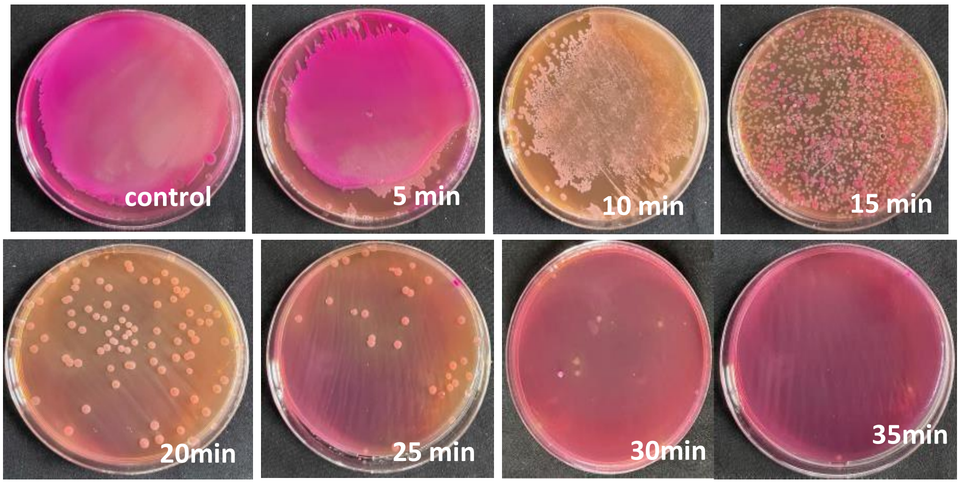

Figure 5: (b) *Escherichia coli* bacteria inactivation at different exposure times.

4. Conclusion

In this study, a non-thermal plasma jet device was built and used to treat *Escherichia coli* bacteria in teeth root canals. The results showed the efficacy of the plasma jet and plasma-activated water (PAW) techniques (PAW). In both methods, as the exposure time was increased, the rate at which the bacteria was killed increased, indicating that time is a significant factor. The treatment with a plasma jet was better, as the complete killing was achieved in a short time, but it took longer to kill bacteria colonies when treated with plasma-activated water. The results also indicated the effectiveness of treatment time for both methods. The obtained results found that the response of bacteria to treatment with plasma jet is better than treatment with plasma-activated water. Still, treatment with PAW has a beneficial effect on reducing the number of colonies, as the plasma produces a high amount of NO_2, NO_3, and H_2O_2 which causes a rapid decrease in the pH value of the activated water indicating the possibility of treating a root canal tooth using

PAW. Further investigation is needed to enhance PAW's antibacterial efficacy and improve the water's activation to shorten the treatment time.

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