In vitro decontamination of infected tooth root canals by single electrode plasma jet

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Abstract: In this study, cold atmospheric pressure plasma generated by single electrode is used to sterilize tooth root canals which were infected with Enterococcus faecalis. The effect of different treatment times with plasma and two types of working gas applying for generating plasma on killing of bacteria is investigated by colony forming unit counting. The optical emission spectroscopy analysis is employed to investigate the active species in the helium and helium/oxygen [1%] plasma jet.

1. Introduction

It is generally believed that the major cause of failure in endodontic is the survival of microorganisms in the apical portion of the tooth [1]. Decontamination of the root canal from the bacteria is the final aim of endodontic treatment which includes cleansing of pulp tissue and removal of bacteria from infected root canals [2]. There is great complexity and variation to the pulp space as it travels from the coronal to the apex [3]. One of the main bacteria which has main role in endodontic failure is E. faecalis [4, 5], commonly detected in asymptomatic, persistent endodontic disinfection. When E.faecalis grows in the root canal system, becomes more resistant, although it can be easily destroyed in vitro.

Conventional treatment methods such as mechanical debridement, chemical irrigation, laser irradiation, ultrasound, and photodynamic therapy cannot attain a complete elimination of biofilms from endodontic sites [6, 7].

For many years plasma has played an essential role in various industries for instance low heat surface modification of polymers [8, 9] clinical instrument sterilization [10, 11], food processing [12], absorption and reflection of electromagnetic radiation [13], so it has become an important part in our lives. Recently plasmas have attracted increased attention in the biomedical field, since non-equilibrium plasmas are able to initiate, promote, control, and catalyze various complex behaviors and responses in biological systems. Some of the plasma applications in medicine is wound healing, blood coagulation, gene transfer to cells [14], cancer therapy [15], and dentistry [16]. As plasma is activated by electrons, the plume emitted generates no heat and can be safely handled.

The aim of this in vitro study is to evaluate the feasibility of disinfection efficiency of atmospheric pressure cold plasma for E. faecalis biofilms in root canal therapy. Early on, the antimicrobial effect of plasma on planktonic pathogens was detected experimentally. Since gaseous properties of plasma, it is applicable in narrow cavities and has an antimicrobial effect against biofilms. Decontamination of plasma with two working gas, Helium and Helium/Oxygen [1%], is investigated. It is found that the mixture working gas, He/O_2, has more effect in bacterial killing. The other parameter studied is treatment time. The disinfection of bacterial load rate gradually increases with the treatment time.

2. Materials and method

2.1. Preparation of root canals

24 single rooted human teeth were selected after extraction and stored in sodium chloride 0.9%, and then all the crowns were removed. The canals were instrumented with hand stainless steel instruments by using a crown-down technique and with physiological saline as irrigant. The root canals were then prepared with hand files up to size #40 (MaillDentsplyefer, Tulsa, OK). The canals were irrigated for 4 min with EDTA to remove the smear layer, and then with hypochlorite sodium 2.25% by vortex. After that teeth apex were sealed by glass ionomer cement (Fugi GC2, Tokyo, Japan). All specimens sterilized in an autoclave at 121 °C for 30 min.

2.2. Bacteria culture

The organisms selected were Enterococcus faecalis (ATCC9854 Iran, Collection of bacteria), which cultured in Brain heart infusion (BHI) broth under aerobic condition at 37 °C. The bacterial concentration used in the experiment was 1.5×10^8 CFU/mL, named 0.5 McFarland standard. The root canals were coated with 0.01 mL of bacterial suspension by injecting it into the canals. For formation the biofilm in the root canals, they were incubated for two weeks.

3. Setup experiment

Fig. 1 shows the schematic of the experimental setup. A single electrode non-thermal atmospheric pressure plasma jet was used to treat in the root canals. The main body of the device is made of a glass tube guiding the gas flow. The copper foil serve as an electrode connected to a high voltage source with 25 kHz frequency and 10 kV pick to pick voltages. The outer and inner electrode diameter of the glass tube is 8 mm and 5 mm, respectively. The nozzle tube diameter is reduced to 1.35 mm. The helium gas with flow rate of 2.6 L/min is used in this experiment as the working gas. Continuous plasma generated in the glass tube, near outer electrode, has a length of 3 cm outside the glass tube. During the treatment with plasma, there is a distance of about 3 mm between plasma jet and the sample. The gas temperature is about 35 °C.

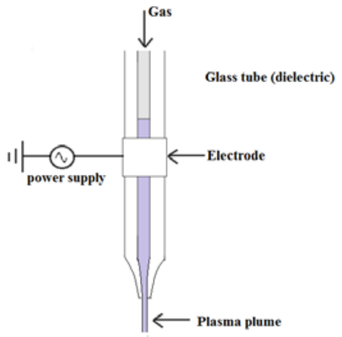

Fig. 1. A schematic diagram of the experimental setup.

It is remarkable that a finger can touch the plasma plume without any feeling of warmth or electric shock; hence the device is safe for biomedical applications such as root canal therapy.

4. Antibacterial treatments of the root canals

After formation of the biofilm in root canals, the excess broth was removed from each well. The samples are devided into 3 goups: first group which contains 3 root canals served as negative control and they are considered as an indicator in order to evaluate external infection in the experiment, second group, positive control, include 3 root canals and received no treatment. The rest of the root canals were put in the third group, which were treated by plasma.

There are two subgroup in the third group. The roots in the first subgroup were treated by helium plasma, and in the second one the mixture gas, helium/oxygen [1%], is used as a working gas for plasma.

In this study tratment time is a parameter which is investigated. For each subgroup various tretment times 6, 8, and 10 minutes is studied.

After plasma treatment canals in each group were washed with sterile saline solution. For each canal, the saline solution was collected with steriled paper points. Then the paper pointd were suspended in 10 mL of sterile saline solution. The K file #40 is used for removing of remaining biofilms in the roots. File is suspended in sterile saline solution, as well and vortexed for 1 minute. For each suspension, serial 10-fold dilutions were prepared.

5. Results and discussion

The viability of the bacteria in the root canals was investigated by colony forming unit (CFU) counting analysis. The results are shown in Fig. 2. No bacteria were detected in any roots in the negative control group. The third group of root canals was designed to determine the effectiveness of plasma jet disinfection applied in this experiment alone in reducing CFU. Reduced CFUs of E. faecalis is observed in all plasma treated groups in compared to positive control group. For treatments with both He and He/O_2 [1%] plasmas, effective growth inhibition of E. faecalis was observed. It can be found that with increasing plasma exposure time, bacterial inactivation degree increased, therefore longer plasma exposure times showed better root canals disinfection. Comparing two working gases to generate plasma in the same treatment conditions showed that the He/O_2 [1%] is more effective than He plasma. It is due to existence of more reactive oxygen species in He/O_2 [1%].

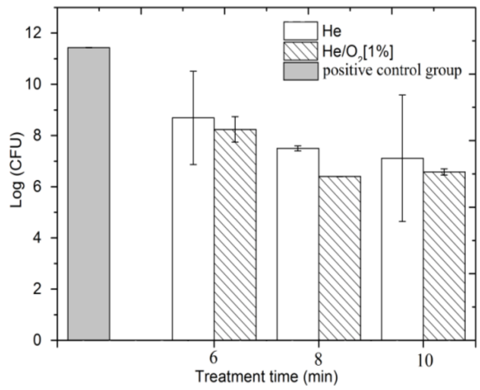

Fig. 2. Microbiology analysis.

6. OES

For identifying various reactive species generated by plasma, which are contributing to the plasma decontamination of bacteria, optical emission spectroscopy (OES) analysis is used. Figs. 3 and 4 show optical emission spectroscopy of single electrode plasma jet used in this experiment with two different working gases. Excited O, O_3, OH, N_2, N_2^+, He are detected in plasma plum. OH is observed in 308.911 nm. At 315.343 nm we can see ozone pick. we consider the antibacterial effect of ozone when it is applied for an adequate time. It is observed that N_2^+ is the highest pick in 390 nm in this analysis. It is well known that atomic oxygen generated when plasma interacts with air, play a crucial role in bacterial inactivation and lead to various biological effects in the intracellular space [17]. Reactive oxygen species (ROS) are able to inactivate cells and cause cell lysis by oxidation [18]. These species act on outer cell membranes and destroy them.

Comparing two generated plasma by helium and helium/oxygen [1%] as working gases illustrated in figure 3 and 4 respectively, shows that using oxygen increases chemical reactive species so the antibacterial effects of plasma improve.

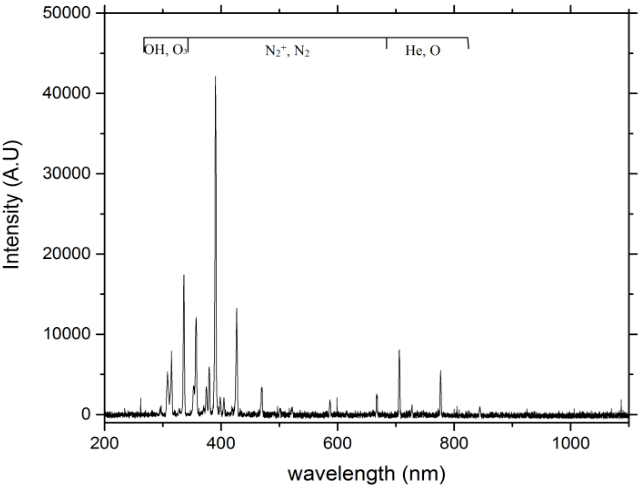

Fig. 3. Emission spectra of the helium plasma.

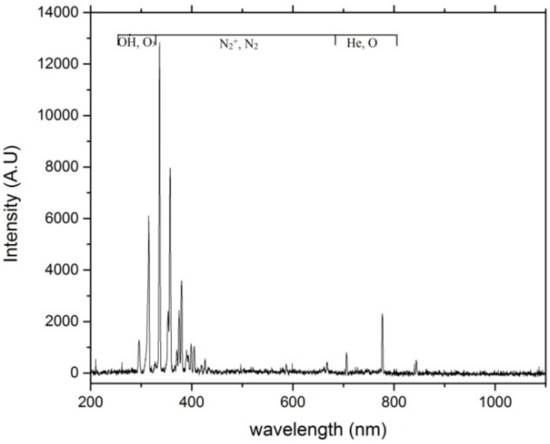

Fig. 4. Emission spectra of the plasma helium/oxygen [1%]

7. Summary and Conclusions

Application of nonthermal plasmas in treatment of root canals is a challenging, multidisciplinary research problem, requiring expertise from both plasma physics and dentistry.

One of the main bacteria cause failure in endodontic treatment is Enterococcus faecalis. It has a high resistance to antibacterial substances and is detected in persistance apical lesions [4, 19]. Therefore safe and effective methods which do not harm tooth structure and surrounding tissuses are necessary.

Cold Plasma Systems are the most common system used in the medical and dental field. It is easy to handle and it has a controlled ozone production rate. Chemically active species such as free radicals, and charged particles play the most important role in the inactivation.

From the CFU counting analysis it is found that plasma with He/O_2 [1%] as the working gas is more effective than He plasma because of more ROS species. ROS play a crucial role in bacterial inactivation. These species act on outer membrain of becteria and kill them.

In this study, the single electrode plasma jet is used to disinfect E. faecalis biofilms in root canals. The plasma temperature was low, so it had no harmful effect on living tissues. Preliminary inactivation experiment results show the plasma tretment can reduce CFUs in E.faecalis biofilms. Increasing the treatment time improve the disinfection degree of E.faecalis biofilm.

8. References

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