Efficacy of cold atmospheric pressure plasma jet against Enterococcus faecalis in apical canal of human single-rooted teeth: a preliminary study

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Abstract. Many factors that may affect the success of root canal treatment are complexity of root canal system, especially in the apical part, and the virulence of bacteria such as biofilm formation. A cold atmospheric pressure plasma jet is a novel method for sterilization and applied for root canal disinfection. The purpose of this study was to investigate the effect of the cold atmospheric pressure plasma jet on Enterococcus faecalis (E. faecalis) biofilms infected in the apical root canals. Methods: Fifty-four single rooted-teeth were infected with E. faecalis for seven days and allocated into five groups. Group I performed as positive control. Group II - V were served as experimental groups: NaOCl, plasma, NaOCl + plasma and gas only, respectively. The disinfection was evaluated by colony count (per milligram) at the depths of 0.05 and 0.10 mm of root dentin. The remaining bacteria was also counted from the grounded root dentin. Data was analysed by one-way ANOVA and post hoc test, with a level of significance set at P<0.05. Results: The NaOCl, plasma and NaOCl + plasma groups significantly reduced the E. faecalis, but no significant difference was found among these groups (P>0.5). The NaOCl + plasma group significantly reduced E. faecalis in the deeper dentin level compared to the other groups. Conclusion: The cold atmospheric pressure plasma jet had antimicrobial activity against E. faecalis biofilm as well as the use of NaOCl. The combined treatment yielded the most promising result and may be beneficial in root canal disinfection.

1. Introduction
The goals of root canal treatment are irritants removal, cleaning and obturation of shaped root canal, and preventing the root canal re-infection. Even though, the conventional root canal treatment is 40-60% reduction of total amount of living bacteria due to not only the complexities of root canal anatomy especially in the apical parts of the roots such as isthmi, apical ramifications and apical deltas, but also microbial in the root canals always form as the biofilms that compose of extracellular polysaccharide (EPS) protecting microbes. The most commonly isolated bacterial species in retreatment cases with apical pathoses are Enterococcus faecalis (E. faecalis) which is able to form the biofilm, attach to the dentinal tubules in the root canals, survive from medicaments i.e. Calcium hydroxide; Ca(OH)₂ (2-5).

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There are several advanced development methods which anticipating for efficiently cleaning the root for example LASERS, nanoparticle, and plasma. Recently, the plasma, a partially ionized gas produced by applying an electric or electromagnetic field to one or more gases\(^6,^7\), is introduced to the biomedical filed including medicine and dentistry. For dentistry, the plasma was studied in many fields such as dental caries, increase bond strength, or root canal disinfection\(^8\). In this in-vitro study, we evaluated the effectiveness of cold atmospheric plasma jet as a disinfection method for the \textit{E. faecalis} biofilm in the apical root canal while different levels of root canal dentin depth were investigated.

2. Materials and Methods

2.1 Preparation of apical root canals
The methods of this study were previously approved by the Human Experimentation Committee, Chiang Mai University, Thailand. Fifty-four single rooted-teeth were cleaned and stored in 0.1% thymol solution before the experiment. The teeth were horizontally cut to acquire the apical root length of 10 mm using diamond discs. Dental pulps were removed. A15 K-file was placed into the root canal and progressed through the canal until it was just visible at the major apical foramen. The working length was recorded as one mm short of this length. The coronal part of root canals was prepared by gates-glidden drill number 2 and 3 at 6- and 8-mm (from the anatomical root apices), respectively. The remaining root canals were enlarged by using nickel-titanium files (Race: FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) to size 40.02. The 5% sodium hypochlorite (NaOCl) was used as an irrigant during the preparation. Each specimen was subjected to final irrigation steps as followed (five minutes each): immersing in 17% EDTA in an ultrasonic bath to remove smear layer, cleaning with 5% NaOCl in an ultrasonic bath and soaking in 3.84% sodium thiosulfate solution to neutralize effects of NaOCl. The apical foramina were sealed with resin composite (Meta Biomed, Seoul, South Korea). Every specimen was submerged into a 50-ml centrifugal tube containing 20 mL of Brain Heart Infusion (BHI) broth. Sterilized all teeth in an autoclave at 121ºC for 15 minutes.

2.2 Enterococcus faecalis biofilm formation
\textit{E. faecalis} (ATCC 29212) was incubated in BHI broth at 37ºC for three hours. The concentration of bacteria was \(1 \times 10^8\) cells/mL measured by an optical density at 600 nm. For the biofilm formation, each canal was filled by 20 µL of BHI broth containing \textit{E. faecalis} and aerobically incubated at 37ºC for seven days. Media in the root canals was changed every two days.

2.3 Cold atmospheric pressure plasma device
A commercial bioplasma jet (Photo Bio Care Co. Ltd, Nonthaburi, Thailand) which employed in skin clinic was used as a power supply for this experiment (Figure 1). The plasma was generated along tungsten wire (length of 1.2 cm and diameter of 0.25 mm) acting as an electrode and 1.7-cm ethylene-tetrafluoroethylene (ETFE) needle (Terumo Surflo®: Terumo Corporation, Tokyo, Japan) as a dielectric barrier. Working gases were helium and oxygen (98% He and 2% O\(_2\)). The flow rate of 0.5 L/min was adjusted by Cole Palmer flow rate meter. The frequency and intensity were set at 10 kHz and 100 Hz, respectively.

![Figure 1](image1.png)

\textbf{Figure 1.}
A photograph of experimental plasma setting (a) and its schematic (b).

2.4 \textit{E. faecalis} biofilm disinfection and assessments
The specimens were randomly divided into controls (N=18) and four experimental groups (N=36). The control groups did not acquire any treatment and were incubated at 37°C for seven days. In the positive control group, the roots were only infected with E. faecalis and there was only BHI broth in the negative control group. For the experimental groups (group 1-4): (1) five mL of NaOCl for one minute, (2) five-minute cold plasma treatment, (3) five mL of NaOCl for 1 minute with 5-minute cold plasma treatment and (4) gas only (He/O2). The plasma tip was placed at 5 mm from the working length. After exposure of each treatment, the root canal dentin was collected by two size files of Race (50.02 and 60.02) into the pre-weighed 1.5-mL Eppendorf tube. The remaining root was freeze in -70 ºC liquid nitrogen and was pulverized. The weighed of each dentin level was determined. The 10-fold serial dilutions were generated and incubated at 37°C for 24 hours. The bacterial concentration was calculated as CFU/mg. The difference between the group was analyzed by one-way ANOVA and post hoc test. P < .05 was considered as statistical difference.

3. Result
Mean log10 CFU data at the level of 50/.02 and 60/.02 for different experimental groups showed that the NaOCl, plasma, and combined groups were effective for reducing the bacterial number (P < .05) and these treatments were more effective than the positive control group and treatment with only working gas (P < .05). Moreover, the combined group inclined more elimination of E. faecalis biofilm in the deeper level of root canal dentin (Figure 2).

![Figure 2. Mean log10 CFU levels in infected root canals after disinfection treatments in different root canal depths.](image)

4. Discussion
Apical periodontitis usually results from an infection in the root canals which bacteria always forms as the biofilms. The biofilm is more resistant to antimicrobial substances than planktonic bacteria(4). The E. faecalis species is commonly chosen as developed models for testing in vitro study designed for endodontic disinfection since it was frequently isolated from the persistent periapical diseases(2-4). Success rate of the conventional root canal treatment is about 68-85%(9). The utilization of cold plasma treatment in Endodontics is to enhance the elimination of root canal bacteria. The reactive species that play an important role in sterilization are low level UV radiation and RONS (reactive oxygen and nitrogen species). The RONS have oxidative effects on the bacterial cell walls so the structure integrity is disturbed(10). Meanwhile, an atmospheric non-thermal plasma or cold plasma as it not only reacts to the bacterial cell membrane leading to ruptured cells and finally cell death, but also causes no or little heat damage to both direct and indirect cell contact(11).

In this study, samples were divided into four experimental groups: NaOCl, cold plasma, NaOCl + plasma and gas only. For the positive control group, E. faecalis formed biofilms attaching to root canal surfaces and some penetrated into dentinal tubules more than a depth of 600 µm. The NaOCl is a widely used irrigant in bacterial disinfection and dissolving tissues during endodontic treatment. The NaOCl groups demonstrated decreasing the number of E. faecalis in every depths of root canal dentin. The cold plasma group showed significant bacterial reduction as well as in the NaOCl and NaOCl + plasma group. The combination of NaOCl and cold plasma showed the most bacterial reduction,
especially in deeper dentin levels. The cold plasma in the gas phase has ability penetrating into deeply root canal system, so the plasma may be able to reach the residue bacteria after using the NaOCl.\(^{(12,13)}\) To increase the success rate of root canal treatment, maximum bacterial elimination before obturation is a key factor\(^{(14)}\). Therefore, the combination of cold plasma with conventional root canal treatment may be used as adjunctive.

For the gas group, the amount of \(E.\ faecalis\) was not significantly different from the positive control group indicating that the He and \(O_2\) alone were not ineffective in killing bacteria without the impulse of electric or electromagnetic fields to break the gas molecules into ions according to previous studies\(^{(15)}\).

5. Conclusion
The cold atmospheric pressure plasma jet had antimicrobial activity against \(E.\ faecalis\) biofilm as well as the use of NaOCl. In addition, the combined treatment yielded the most promising result and may be beneficial in root canal therapy.

6. References
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