Application of Non-Thermal Plasma As An Endodontic Therapy For E. faecalis Biofilm Infected Tooth Root Canal

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Abstract: E. faecalis grow within dentinal tubules, re-inflect the obturated root canal and cause endodontic failure, which is the major reason for the failure of treatment of endodontic. Moreover, the biofilm of E. faecalis which have strong canitbiotic-resistance than planktonic bacteria, can also cause life-threatening infections in humans, especially in the nosocomial (hospital) environment. In this study, we demonstrate a promising method for disinfection of the biofilm of E. faecalis by an atmospheric-pressure Ar/O2 (2%) cold plasma microjet (PMJ). The inactivation of the biofilm of E. faecalis was verified. The assessment of re-infection of E. faecalis was evaluated by the comparison of the bactericidal efficiency between the plasma and the clinical chemical. SEM (Scanning electron microscopy) and SEM/EDS (Scanning Electron Microscopy with X-ray microanalysis) were employed to evaluate the plasma effect on the biofilm and tooth structures. Reactive oxygen species were detected by Electron Spin Resonance (ESR) spectroscopy in liquid and optical emission spectra in air. Possible pathway for the disinfection will be discussed.

Keywords: Non-thermal Plasma, E. faecalis Biofilm, Tooth Root Canal, Electron Spin Resonance

1. Introduction

Bacteria infection has long been recognized as the primary etiologic factor in the development of pulp and periapical lesions [1]. Many studies have demonstrated that persistent endodontic infections are frequently caused by E. faecalis [2]. E. faecalis can be easily destroyed in vitro, but it becomes more resistant when grow in the root canal system [3]. It possesses some proteases could help it bind to dentin,which including serine protease, gelatinase, and collagen-binding protein (Ace) [4]. Because of E. faecalis biological properties, it has the capacity to live in dentinal tubules and endure prolonged times of starvation [5,6]. The biofilm of E. faecalis which have strong canitbiotic-resistance than planktonic bacteria, can also cause life-threatening infections in humans. The biofilm bacteria become 1000-fold more resistant to phagocytosis, antibodies, and antimicrobials than planktonic cells. [7,8]. The purpose of root canal treatment is to eliminate the infection of the root canal system and prevent re-infection. For treatment of root canal infection, the primary methods include mechanic cleaning, irrigation, laser irradiation, ultrasound, and the application of hypochlorite and other antibacterial compounds [9]. Standard intracanal antisepsis usually can not achieve a complete elimination of microorganisms biofilm from endodontic sites [10]. In fact, some studies have demonstrated that the incidence of negative cultures ranges from 40%–60% of the cases treated by those standard intracanal antisepsis strategies [11]. For instance, calcium hydroxide, which is an inter-appointment intracanal medicament routinely applied in clinic endodontic disinfection treatment, but E. faecalis in dentinal tubules has been witnessed to resist it about over ten days[12,13]. It has limited effectiveness in eliminating bacteria biofilm from human root canal
[14,15]. In these cases, to eradicate *E. faecalis* biofilm from persistent root canal infections, more effective disinfection methods are highly expected for endodontic therapy.

Recently, atmospheric-pressure non-thermal plasmas emerged as a novel tool in dentistry [16,17,18], especially in tooth root canal disinfection [19,20,21,22]. E. Stoffels and co-workers [22] reported that non-thermal plasmas do not cause significant thermal burn or destruction. They also discussed the probability of applying non-thermal plasmas in the oral environment. Lu and co-workers [19] reported preliminary results on a real infected tooth root canal with a He (O2) plasma plume driven by a high-voltage (HV) submicrosecond pulsed direct-current (DC) power supply. Root canals were not completely disinfected in that experiment with 10 minutes of plasma treatment. Jiang and co-workers developed a He (O2) plasma plume [21] that was powered by 4–6-kV ~100-ns electric pulses at a repetition rate up to 2 kHz. They stated a highly promising capability of these plasmas in endodontic disinfection. However, SEM results revealed that plasmas failed to reach below 1 mm in depth where biofilms were still present. Lately, R. Bussiahn and co-workers [23] reported the generation of a hairline plasma with a length up to 1.5 cm, which could be adjusted to treat the tip of the root canal. Despite the variation of the devices, preliminary results from these groups show effective disinfection of root canal on extracted tooth or tooth models in a time ranging from 4 minutes to 5 minutes. However, to our best knowledge, no study has shown to eradicate *E. faecalis* biofilm completely and assessment on re-infection.

In this study, we used a Ar/O2 (2%) plasma jet to disinfect *E. Faecalis* biofilm which was incubated in tooth root canal. Re-infection of the bacterial in tooth root canal was evaluated and compared with clinical therapy, such as formocresol and calcium hydroxide.

**2. Materials and Methods**

50 Single-rooted extracted human teeth were stored at 4°C in 1% thymol solution before experiment. All tooth specimens were decoronated under the cemento–enamel junction (CEJ) leaving a standard tooth length of 10 mm. The root canals were then prepared with Ni–Ti hand files up to size #40 (Mani. Inc., Japan). Each apical foramen was sealed with composite resin (Clearfill AP-X, Kuraray Dental, Japan). All specimens were sterilized by an autoclave before further treatment. *E. faecalis* (ATCC29212) was cultured in 1ml BHI medium for 1 days. The experiments were divided into two parts, as shown in Table 1. Part I evaluates the effectiveness of the PMJ on disinfection of root canals. Group A was treated with gas flow (without plasma) for 2, 4, 6 and 8 minutes, respectively and used as negative control. Group B was treated by PMJ for the same time periods. Part II of the experiments evaluates the re-infection of *E. faecalis* after PMJ treatment. Samples in groups were treated with clinical medicaments for 1 days: Group D with Ca(OH)2. Group E was treated with PMJ for 10–40 minutes (10 minutes increment).

**3. Results**

**Root canal disinfection**

Results showed that the disinfection rates of *E. faecalis* biofilm in Group B with various PMJ treatment times. Clear reduction of the bacterial colonies was observed after a 2-minute plasma treatment. The disinfection rate gradually increases with the treatment time and reaches 98.8% after an 8-minute PMJ treatment. Three teeth treated with PMJ for 8 minutes were also evaluated for re-infection following the plasma treatment. The disinfection rate drops to about 60%, which indicates the re-infection in the obturated root canal after 7 days. This is likely due to the complex structure of human tooth root canal — the bacteria located in areas such as isthmuses, ramifications, deltas, irregularities, and dentinal tubules are rather difficult to be eliminated [23].

Root canal treated with PMJ for 10 minutes was re-infected after 7 days, showing a re-infection rate of about 33%. The re-infection rate decreases gradually with the increase of the PMJ treatment time, reaching 0.8% for that with 30-min PMJ treatment. The samples treated with PMJ for 40 minutes present no re-infection, while the samples treated
with clinical medications for 7 consecutive days show re-infection rate of 4.2% (Ca(OH)\textsubscript{2}). The details of the results about inactivation of 7-days biofilm and evaluation of re-infection will be further discussed on the conference.

**SEM evaluation**

In the group A, *E. faecils* biofilm covered the entire root canal surface from the top third to apical third. (Figure 1 (a), (b) and (c)). By 8-minute plasma treatment, the root canal is partially cleaned with a clear contrast line separating the zones with and without bacteria (arrow in figure 1(d)), indicating the active species generated by plasma have a limited penetration depth in 8 minutes. The middle third of the tooth root canal with open dentinal tubules (microscopic channels leading to tooth nerve center – pulp) is predominantly cleaned with no obvious bacteria observed around dentinal tubules’ openings (figure 1(e)). A considerable amount of bacteria still survived in the apical third of the root canal (Figure 1(f)). When extending the treatment time to 30 minutes, the entire root canal (from basal to apical) was thoroughly cleaned with no obvious bacteria observed in SEM pictures (figure 4 (g), (h) and (i)).

**Electron Spin Resonance (ESR) Spectroscopy**

The PMJ was sustained in a quasisteady gas cavity in water for ESR diagnosis. Hydroxyl radical \( \cdot OH \) and \(^1\text{O}_2\) detected in the PMJ-water system. When DMPO was added into 1 ml distilled water and treated with PMJ for 20 seconds, quartet DMPO-OH adduct spectrum was observed. TEMP was used to spin trap singlet molecular oxygen \((^3\text{O}_2)\). Typical triplet TEMPO signal in ESR spectrum is shown (aN=1.72 mT), indicating the existence of \(^3\text{O}_2\) in the plasma-liquid system.

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**References**


Figure 1. LEFT: an illustrative picture of human tooth. The three boxes show three locations (root canal, the middle third and the apical third of the root canal) where images are taken. RIGHT: SEM images of *E. faecalis* biofilm infected tooth root canal from Group A: (a)(b)(c), Group B (8 minutes of PMJ treatment): (d)(e)(f) and Group G (30 minutes of PMJ treatment): (g)(h)(i).

<table>
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<th>Part I</th>
<th>Group</th>
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Table 1. A list of the treatment groups