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Antimicrobial effects of non-thermal atmospheric plasma as a novel root canal disinfectant

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ABSTRACT

This study aimed to investigate effects of non-thermal atmospheric plasma on an *Enterococcus faecalis* biofilm within the canals of extracted human teeth. A significant decrease in the number of CFU's was observed after 2 min cold plasma treatment with an average kill rate of 99.999%. MTT assay showed a significant reduction in the viability of bacteria with a reduction rate of 98.939%. XTT assay showed a reduction of bacterial metabolic activity by 99.7%. Both 2 min cold plasma and 6% NaOCl greatly reduced the viability and metabolic activity of *E. faecalis* bacteria, but there is no significant difference between them.

1. Introduction

Success of endodontic treatment depends on complete elimination of the infection in root canal system and prevention of reinfection [1]. Thus, complete disinfection of an infected root canal is critical before obturation. However, this remains a clinical challenge because by traditional methods, such as mechanical debridement, chemical irrigation, laser irradiation, and ultrasound, it is difficult to achieve complete disinfection due to the complexity of the root canal system [2]. Studies have shown that the remaining bacterial culture could range from 40% to 60% in those standard intracanal disinfection strategies [3,4].

Non-thermal plasmas, or low-temperature gas plasmas, are partially ionized gases that contain highly reactive particles including electronically excited atoms, molecules, ionic and free radical species, while the gas phase remains near room temperature. Cold plasmas combine exceptional chemical activity with relatively mild, non-destructive character due to the near room temperature gas phase. Because the gas phase can be controlled at or close to room temperature, plasma treatment of a biological system is not due to heat but the controllable plasma reactive species. The treated substrate surfaces do not suffer any thermal damage. Many research studies have shown that non-thermal atmospheric plasmas are very effective and efficient in destruction/disinfection of bacteria [5–8]. Recently several compact size atmospheric plasma sources have been successfully developed [9–11]. These novel atmospheric plasma sources create non-thermal plasmas with miniature sizes and well-controlled gas temperature. The emergence of these novel miniature plasma sources has inspired the plasma research into biomedical applications. Our research team has studied cold plasma for dental applications using a miniature compact size atmospheric cold plasma brush (ACPB) developed by the Center for Surface Science and Plasma Technology (CSSPT) at University of Missouri. Our study showed that cold plasma could increase chemical interaction of dentin substrate with dental adhesives after 30 s plasma treatment of the dentin surfaces [12]. Our other study showed cold plasma highly effective in killing cariogenic oral bacteria. The plasma exposure time for a 99.9999% cell reduction was less than 15 s for *Streptococcus mutans* and within 5 min for *Lactobacillus acidophilus* [13].

Many studies have shown that persistent endodontic infections are frequently caused by *Enterococcus faecalis* [14]. Previous studies indicated that *E. faecalis* has the capacity to live in dentinal tubules for prolonged time and become more resistant when growing in the root canal system, especially when *E. faecalis* biofilm is developed [15–19]. In this in-vitro study, we evaluated the effectiveness of non-thermal atmospheric cold plasma as a noval method for in vitro root canal disinfection using extracted

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human teeth. *E. faecalis* was used to infect the root canal and develop biofilm.

2. Methods and material

2.1. Preparation of root canal

The University of Tennessee Health Science Center (UTHSC) Institutional Review Board (I.R.B.) reviewed and approved this study. Twenty-four anterior single-rooted human teeth were extracted and collected from the UTHSC College of Dentistry, which were then divided into three groups. The crown of each tooth was reduced with a slow-speed handpiece and dental burs. The remaining tooth structure was trimmed to a root length of 10–12 mm. All roots were instrumented with a standard endodontic technique and irrigated with 5 ml 15% ethylenediaminetetraacetic acid (EDTA) to a final canal size in range from a 45 file to a 60 file. After standard instrumentation was completed, the apical orifice was sealed with dental wax. Then the canals were dried and autoclaved (Fig. 1A).

2.2. Low temperature atmospheric argon plasma brush

The detail of this plasma source was described previously [20]. Argon (99.99% purity) gas was used as the main plasma gas (30 standard liter (SL)/minute) and oxygen (99.99% purity) as the additive gas (30 standard cubic centimeter (SCC)/minute). MKS mass flow controller (MKS Instruments Inc. Andover, MA, USA) was used to control argon gas flow rate. The discharge was ignited and sustained by a DC power supply (Pd 1556c, Power Design Inc. New York, NY, USA). With a relatively high gas flow rate, which varies with the plasma chamber size, the plasma discharge formed inside the chamber is blown out of the chamber. When blown out of the chamber, it formed a brush-shaped low temperature plasma jet. The plasma source can be operated under very low electrical power (as low as a few watts), therefore allowing low plasma temperatures to be achieved. The gas phase temperatures of argon atmospheric plasma, which were measured using an infrared camera combined with a thermocouple thermometer ranged from 30 °C to 65 °C, with the corresponding argon gas flow rate varying from 500 sccm (standard cubic centimeter/minute, a volumetric flow rate at 273 K under 1 atm) to 3500 sccm and input power from 5 W to 15 W (Fig. 2).

2.3. Bacterial growth

E. faecalis (ATCC 29212) was cultured overnight at 37 °C in 10 ml tryptocase soy broth (TSB). Bacteria was harvested by centrifugation and re-suspended in 2 ml phosphate buffered saline (PBS). The bacterial suspension was adjusted to contain 1×10^9 cells/ml by measuring optical density at 600 nm. The bacteria were then diluted to contain 1×10^8 bacteria in 60 μl.

2.4. Root canal biofilm

The bacterial biofilm was formed by injecting each of the twenty-four canals with a highly concentrated bacterial suspension of *E. faecalis* (Fig. 1B). Infected root canals were incubated at 37 °C for 48 h in a sealed humidified chamber. Following the incubation, the teeth were divided into three groups. Group (1) was flushed three times with sterile media PBS and plated on TSB agar plates. The media was collected and plated on blood agar plates to enumerate the viable bacteria. Group (2) was similarly flushed with 6% sodium hypochlorite, which is the standard antibacterial agent for endodontic treatment. Group (3) was subjected to plasma treatment for 2 min (Fig. 1C). The root canals were injected with 0.5 ml of media, followed by counting the number of viable bacteria. Samples from each tooth were diluted appropriately in PBS and plated on TSB agar plates. Plates were incubated at 37 °C for 48 h, and then bacterial colonies were counted.

2.5. Measurement of viable bacteria by MTT viability assay

Anti-bacterial effects of non-thermal plasma on cell viability was assessed by determining the ability of bacteria to cleave the tetrazolium salt (MTT) to a formazan dye, using a kit from Boehringer Mannheim Corp. (Indianapolis, IN, U.S.A). Following the treatment of root canals, the PBS wash (150 μl) was incubated with MTT reagent (final concentration of 0.5 mg/ml) for 4 h at 37 °C. Purple formazan crystals produced from the MTT by

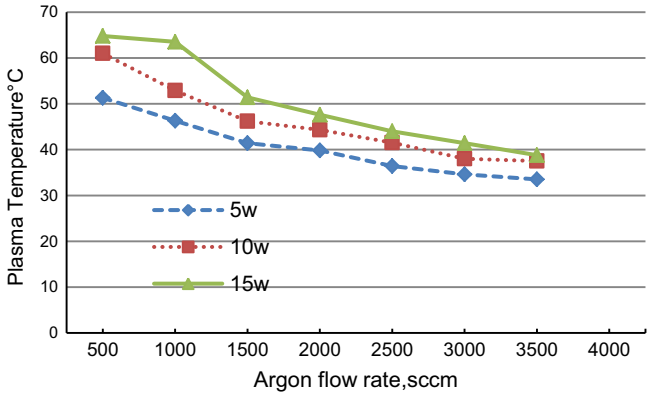

Fig. 2. Temperature change of argon atmospheric plasmas with argon flow rate at different DC power input.

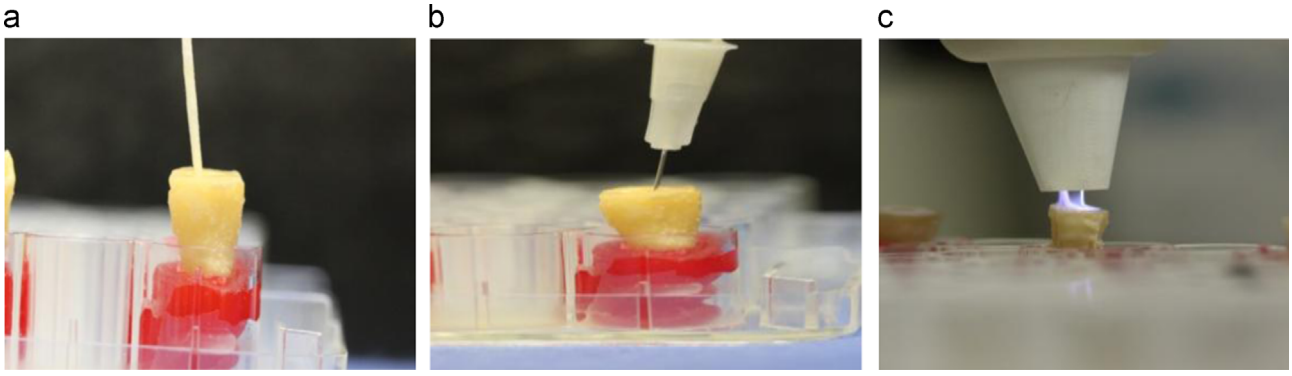

Fig. 1. (a) Root canal was instrumented, dried and autoclaved, (b) bacterial suspension was injected and incubated, and (c) root canal was treated with cold plasma for 2 min.