

Low-Pressure Radiofrequency Cold Plasma for Disinfection of Gutta-Percha Cones

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ABSTRACT

Background and Aim: Different methods have been proposed for rapid disinfection of gutta-percha (GP) cones. This study aimed to assess the efficacy of low-pressure radiofrequency cold plasma (LRFCP) in disinfection of GP cones compared to three chemical disinfectants.

Materials and Methods: Seventy GP cones were allocated to seven groups of 10 each. All samples were initially sterilized with ethylene oxide (EO) and subsequently inoculated with *Staphylococcus aureus* (*S. aureus*), except for the negative control group (n=10). In the experimental groups (n=50), samples were subjected to one-minute chemical disinfection [5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), and 10% Deconex® 53 PLUS) or LRFCP (30 seconds or one minute). The effectiveness of disinfection was evaluated by counting the colony-forming units (CFUs). Data were analyzed using Kruskal-Wallis test (P=0.05).

Result: All experimental groups effectively eliminated *S. aureus*. LRFCP and 5.25% NaOCl were the most effective agents in disinfection of GP cones. In addition, 2% CHX was significantly weaker than the other agents (P<0.05). Although Deconex® 53 PLUS was less potent than LRFCP groups and NaOCl (P>0.05), it showed higher antibacterial activity than 2% CHX (P>0.05).

Conclusion: LRFCP can be assumed as a noninvasive and efficient method for disinfection of GP cones.

Keywords: Disinfection, Gutta-Percha, Cold Plasma, *Staphylococcus aureus*

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Introduction:

The main objective of endodontic obturation is to hinder the recontamination of a prepared root canal space by the oral microbiota.⁽¹⁾ Pulp and periapical pathoses are directly related to the presence of microorganisms.^(2,3) Thus, elimination or massive reduction in the number of exogenous or endogenous microorganisms throughout endodontic treatment should be regarded as the fundamental concept to achieve a successful outcome.^(4,5)

Gutta-percha (GP) cones are commonly used for root canal filling. They might become contaminated by pathogens during handling and storage in clinical use. Hence, many authors have recommended chair-side disinfection of GP cones before use.⁽⁵⁻⁸⁾ Since GP has thermoplastic characteristics, it cannot undergo heat sterilization. However, different chemical agents have been proposed for rapid chemical disinfection of GP cones.^(5,9) Some physical changes have been reported after such procedures.⁽¹⁰⁻¹²⁾

Plasma, a partially or fully ionized gas often referred to as the fourth state of matter, features quasi-neutrality and collective behavior.⁽¹³⁾ The sterilizing effect of plasma was reported by Menashi for the first time in 1968.⁽¹⁴⁾ Efficient inactivation of different types of pathogens, including vegetative bacteria, bacterial endospores, fungi, viruses, prions, and endotoxins, has been reported for non-thermal/cold plasmas.⁽¹⁵⁾ To date, there has been no research on the efficacy of cold plasma for decontamination of GP cones. Therefore, this study aimed to compare low-pressure radiofrequency cold plasma (LRFPC) with rapid chemical sterilization techniques [5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), and 10% Deconex® 53 PLUS] for decontamination of GP cones inoculated with *Staphylococcus aureus* (*S. aureus*).

Materials and Methods:

To evaluate LRFPC for disinfection of GP cones, 70 #80 GP cones were sterilized with ethylene oxide (EO). In addition to positive (n=10) and negative control (n=10) groups, five groups of 10 samples each were designated to receive one of the following protocols: LRFPC (60W, 30 seconds), LRFPC (60W, one minute), NaOCl (5.25%, one minute), CHX (2%, one minute), and Deconex® 53 PLUS (10%, one minute). Negative controls were used for assessment of the efficacy of the initial EO sterilization process. Positive controls were used for obtaining the baseline colony-forming unit (CFU) count as well as confirmation of bacterial growth on the samples throughout the study. *S. aureus* (ATCC 25923) was streak-cultured from the frozen stock on a nutrient agar plate (Merck, Germany) and incubated at 37°C for 24 hours. A bacterial concentration compatible with 0.5 McFarland standard was prepared from freshly cultured bacterial colonies and diluted with 0.9% sodium chloride (NaCl) to obtain the final microbial suspensions at the concentration of 1-2×10⁶ CFUs/ml.

Contamination of GP cones in the experimental and positive control groups:

Every five samples, regardless of being experimental or control, were inserted into one sterile Eppendorf tube containing one ml of the aforementioned microbial suspension and left for 30 minutes to promote surface adsorption. Thus, in total, 12 Eppendorf tubes were used for bacterial inoculation of positive and experimental groups.

These contaminated GP cones were transferred to petri dishes that had been matted with two layers of sterile filter paper and placed in a dry heat sterilizer for 30 minutes at 37°C until they were dry. Each sample was then placed in a sterile Eppendorf tube filled with one ml of sterile nutrient broth and incubated at 37°C for 48 hours. A table of random numbers was used for the allocation of samples to the groups.

Chemical protocols:

The agents used for chemical disinfection were 5.25% NaOCl (Cerkamed, Poland), 2% CHX (Coltene, Switzerland), and 10% Deconex® 53 PLUS (Borer Chemie AG, Switzerland). Each inoculated sample was immersed in one of these agents for one minute. Before returning each GP to a new Eppendorf tube containing 1ml sterile nutrient broth, sterile saline bath was used for washing the chemical residues.

Cold plasma sterilization:

An RF plasma reactor (13.56 MHz, 60W), a research reactor designed by the School of Advanced Technologies in Medicine at Shahid Beheshti University of Medical Sciences, Tehran, Iran, was used for either 30-second or one-minute exposure of allocated samples. The volume and the pressure of the chamber were 1000 cm³ and 0.1 mbar equal to 10 Pascals (Pa), respectively. Each sample was then immediately transferred to a new Eppendorf tube containing 1ml of sterile nutrient broth.

After 10 minutes of immersion, all tubes were vortexed and the broth was subjected to 2-fold serial dilution; 100 µl of each dilution was transferred to a nutrient agar plate and incubated at 37°C for 48 hours. Thereafter, CFUs were counted for each GP cone; microbial detection limit was determined to be three CFUs. Since the distribution of the data was not normal, reduction in the number of colonies was analyzed with Kruskal-Wallis test using SPSS software (version 22, SPSS Inc., Chicago, IL, USA). The level of significance was set at 0.05. Multiple comparisons were made using Dunn's multiple comparison test.

Results:

Kruskal-Wallis test was used for statistical analysis ($\alpha=0.05$). After the first 48 hours, all positive controls showed positive results whereas negative controls demonstrated no microbial growth, inferring effective initial EO sterilization. All methods showed significant antibacterial activity against *S. aureus* in comparison with the positive control group ($P<0.001$). LRFCP and 5.25% NaOCl were significantly more effective than 2% CHX (P-values for 30-second LRFCP, one-minute LRFCP groups, and NaOCl were 0.003, 0.001, and 0.003, respectively). The difference between LRFCP and 5.25% NaOCl was not significant ($P>0.05$).

Deconex® 53 PLUS was insignificantly more potent than 2% CHX and less effective than LRFCP and 5.25% NaOCl ($P>0.05$). Moreover, 2% CHX was significantly weaker compared to the other three experimental groups ($P<0.05$). Although Deconex® 53 PLUS was less potent than LRFCP groups and NaOCl ($P>0.05$), it showed higher antibacterial activity than 2% CHX ($P>0.05$). Table 1 shows the data related to different study groups.

Figure 1 demonstrates the mean rank values for the experimental groups.

Table 1. Colony-forming unit (CFU) counts for the experimental and positive control groups (n=10).

| Groups | Minimum | Maximum | Mean | SD | Sterilization (%) |
|--------------------|---------|---------|------|-------|-------------------|
| LRFCP (30 seconds) | 0 | 1 | 0.2 | 0.42 | 80 |
| LRFCP (one minute) | 0 | 1 | 0.1 | 0.31 | 90 |
| Deconex 53PLUS | 0 | 2 | 0.8 | 0.79 | 40 |
| 5.25% NaOCl | 0 | 1 | 0.2 | 0.42 | 80 |
| 2% CHX | 0 | 3 | 1.7 | 0.94 | 10 |
| Positive Control | 21 | 224 | 62.3 | 64.90 | N/A |

CHX=chlorhexidine, LRFCP=low-pressure radiofrequency cold plasma, NaOCl=sodium hypochlorite, SD=standard deviation, N/A=not applicable

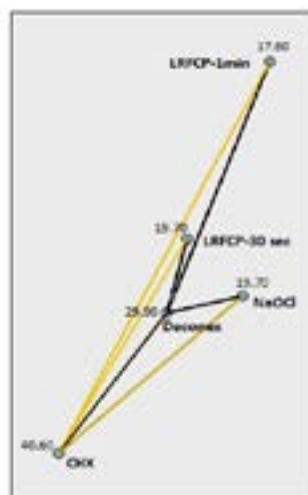


Figure 1. Mean rank values for the experimental groups. CHX=chlorhexidine, LRFCP=low-pressure radiofrequency cold plasma, NaOCl=sodium hypochlorite.

Discussion:

The goal of this study was to compare the antibacterial effectiveness of LRFCP with that of rapid chemical technique using 5.25% NaOCl, 2% CHX, and 10% Deconex® 53 PLUS for decontamination of GP cones inoculated with *S. aureus*.

The ultimate goal in endodontic treatment is to reduce the bacterial load as much as possible to provide an environment conducive to healing.⁽¹⁶⁾ In this regard, aseptic practices, including sterilization of armamentarium and decontamination of the operative field and materials, are crucial. GP cones are very popular as an obturating material in endodontics. They offer thermoplasticity, tissue tolerance, dimensional stability, and radiopacity. Moreover, they can be readily removed in case of endodontic retreatments. Since the placement of GP is the final step of obturation of a prepared root canal, the clinical condition of GP cones is a matter of concern. Despite the inherent antibacterial properties of GP, which have been mainly attributed to its zinc oxide component,^(4,17,18) many studies have shown the presence of bacteria on commercial GP cones in sealed boxes or shortly after exposure to the clinical environment.^(8,19-21) Because GP is heat-labile, a wide array of chemical disinfectants have been proposed to disinfect GP cones before root canal obturation.^(5-8,19,21-24) However, some physical changes in GP cones after such procedures seem to be unavoidable.^(10-12,25) Gamma irradiation, a nonchemical approach for heat-sensitive materials, also poses some problems including time-dependent physical and chemical damage.^(26,27) Plasma, a partially ionized gas, has several anti-pathogenic properties.^(15,28-36) Inactivation of microorganisms by plasma is strongly related to generated electrons and ions. Because of the high flux and penetration depth of electrons, they induce the formation of reactive oxygen species (ROS) and strong oxidants in contact with water. Similarly, the chemical effect of ions can generate highly reactive oxidants. Moreover, ion bombardment can cause cell membrane damage by mechanical impact.⁽²⁸⁾

In this study, RF plasma was used for disinfection of GP cones because these plasmas are characterized by displacement current, inferring that charges only oscillate within the electric field

without flowing to the electrodes; this allows the electrodes to be placed outside the low-pressure compartment, resulting in reliability, reproducibility, and increased lifetime of the reactor.⁽³⁷⁾ LRFCPs have been used for processing of fibers, biomedical and microfluid devices, polyacrylate/silica nanocomposites, and decontamination of endodontic files.⁽³⁸⁻⁴³⁾ In the present study, we used *S. aureus* for inoculation of GP cones. The rationale behind this was the fact that the main contaminating bacteria present on GP cones are vegetative bacterial cells (*Staphylococcus* spp.) rather than resistant spores.^(21,25) Thus, the use of arbitrary resistant or spore-forming flora as target microorganisms may not be highly clinically relevant.⁽⁹⁾ *Staphylococci* are the normal flora of the skin and mucous membranes with the ability of extracellular polysaccharide synthesis, initiating biofilm formation.⁽⁴⁴⁾ It has been proposed that the biofilm formed on overextended GPs might be related to refractory periapical pathoses.^(45,46) Based on the finding of this study, LRFCP treatments were equally efficacious to 5.25% NaOCl. However, immersion of GP cones in 5.25% NaOCl, known as rapid sterilization technique initially proposed by Senia et al,⁽⁵⁾ has been frequently suggested as an efficient, reliable, convenient, and inexpensive method of GP decontamination.^(5-7,21,23) There are some concerns regarding chemical deterioration of GP and chloride precipitation^(25,47,48) its aggressiveness towards periapical tissues,^(21,23,47) as well as difficulties with fresh preparation of the solution and its chemical stability.⁽⁴⁹⁾ While different methods have been recommended for elimination of residual crystals forming,^(47,50) they increase the time needed for clinical use, negatively affecting the reported efficiency for the original technique. In contrast, RFCP used in this study has no toxic residual effect and reduced turnover time.^(51,52) In CP, the temperature of electrons is considerably higher than that of heavy particles, and the electron density is normally below 10^{19} m^{-3} . As heavy particles determine overall temperature, a CP simultaneously serves the purpose of high reactivity using energetic electrons and low temperature due to low-energy heavy particles.⁽⁵³⁾ In this study, the pressure of the chamber was first lowered to further promote electron acceleration rather than heavy particle collisions.⁽⁵⁴⁾ Secondly,

RF was used to guarantee electron stimulation instead of ion oscillation.⁽⁵⁵⁾ Thus, elevated temperature can be readily ruled out as a major phenomenon in bacterial inactivation. Concern over RFCP is that the complex surface structure such as pits, cracks, and grooves can shield pathogens from reactive species and thus impede microbial inactivation.^(35,55,56) According to the finding of the present study, this is not the case for GP cones as they do not have complex geometry where microorganisms can hide; therefore, LRFCP was as effective as NaOCl.

Although some studies have shown a single slope exponential microbial decay, reflecting equal exposure of a homogenous culture to identical conditions or predominance of elevated temperature as major inactivation effect for plasmas,^(32,33,55) the present study revealed multi-slope microbial inactivation characteristics and biphasic curves. This finding is in line with most studies on plasma decontamination.⁽³⁴⁻³⁶⁾

Conclusion:

Within the limitations of this study, LRFCP can be considered as a valuable tool for disinfection of GP cones.

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