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Ex vivo evaluation of the efficacy of depotphoresis method in root canal disinfection

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ABSTRACT

Background: Electrochemical disinfection of the root canal system (RCS) is introduced as an alternative to conventional irrigation. The aim of this study was to assess the efficacy of depotphoresis method in the disinfection of accessible and inaccessible RCSs.

Materials and Methods: In this comparative *in vitro* study disinfection of *Enterococcus faecalis*-infected RCS using two methods, (1) depotphoresis and (2) sodium hypochlorite (NaOCl) irrigation plus passive ultrasonic agitation (PUA) took place on 40 extracted maxillary anterior teeth. Decoronation was done with a diamond disc, and the canals were instrumented. The roots were divided into two phases: the specimens with canal obstruction and the specimens without canal obstruction. The smear layer was removed, and the specimens were infected for 21 days with *E. faecalis*. After disinfection procedures, bacterial samples were taken using two sterile #35 paper points, and colony-forming units (CFU) were counted. Data were analyzed statistically using the Kruskal–Wallis test, with a significance level at $P < 0.05$, to indicate differences between depotphoresis and NaOCl plus PUA groups.

Results: In both phases, Log CFU after depotphoresis treatment was significantly lower than NaOCl irrigation plus PUA treatment ($P < 0.05$).

Conclusion: Treatment with depotphoresis was significantly more effective than NaOCl irrigation plus PUA treatment.

Key Words: Disinfection, *Enterococcus faecalis*, iontophoresis, root canal irrigants, sodium hypochlorite

INTRODUCTION

The microbial causation of apical periodontitis has been well-documented.[1,2] Dentinal tubules and apical irregularities of root canal anatomy such as deltas and isthmuses may harbor tenacious biofilms, contributing to the failure of endodontic treatment.[3] Conventional mechanical instrumentation and irrigation with sodium hypochlorite (NaOCl) would not predictably make root canals free of bacteria.[4,5]

Calcium hydroxide (CH), as an interappointment medicament, reduces bacteria in dentinal tubules.[6] However, a systematic review concluded that CH has

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limited antibacterial effectiveness,[7] and the remnants of it can affect the sealing ability of root canal sealers.[8]

Knappwost has introduced a technique named depotphoresis for the disinfection of root canal system (RCS) using CH with copper ions (cupral).[9] Cupral is placed in the coronal third of the RCS, and the ions are activated and moved to the apical third of the RCS by electric current. It has been shown that depotphoresis eradicates viable bacteria in dentinal tubules to a depth of 500 μm.[6,10] It has been claimed by the manufacturer of the depotphoresis system that "the electrical field transports cupral even in the mechanically not reachable apical part of the canal."[11]

The purpose of this study was to evaluate the efficacy of depotphoresis method in the disinfection of accessible and inaccessible RCS.

MATERIALS AND METHODS

The methods of this *in vitro* study were approved by the Research Ethics Committee of Mashhad University of Medical Sciences (Approval ID: IR.MUMS.DENTISTRY.REC.1399.027).

Forty freshly extracted noncarious mature maxillary anterior teeth were collected and kept in saline. After placing the teeth in 5.25% NaOCl for 30 min to detach residual debris from the root surfaces, a diamond disc was used to cut the crowns perpendicular to the long axis of the teeth to have the same length of roots (16 mm). A #10 K-type file was placed into each root canal to confirm apical patency.

Then, the roots were divided into two phases: the specimens without canal obstruction and the specimens with canal obstruction.

Phase 1:The specimens without canal obstruction

The root canals were instrumented to F3 (ProTaper Universal; Dentsply Sirona, Ballaigues, Switzerland) 0.5 mm beyond the apical foramen. Irrigation with 5.25% NaOCl was performed between each file insertion. To remove the smear layer, a final irrigation sequence of 5 ml of 17% EDTA for 5 min, 5 ml of 5.25% NaOCl for 5 min, and 10 ml of normal saline was done according to Lin *et al.*'s study.[10] The roots were placed in Eppendorf tubes containing 2 ml of Tryptic Soy Broth (TSB) medium and then autoclaved for 30 min at a pressure of 15 PSI and temperature of 121°C. Then, the tubes were incubated at 37°C for 24 h. A sterile micropipette was used to aspirate TSB and deliver 1 ml of *Enterococcus faecalis* suspension with a standard concentration of 0.5 McFarland (1.5×10^8 colony-forming units [CFU]/ml) into the root canal and around the root so as to immerse the root in the *E. faecalis* suspension. Thereafter, the tubes were incubated at 37°C for 24 h, and bacterial viability was determined by CFU count. Subsequently, the specimens were incubated at 37°C for 21 days. To prohibit dehydration of the specimens during incubation, the TSB medium was refreshed every 3 days.

The roots were randomly divided into NaOCl plus passive ultrasonic agitation (PUA) group (n = 10) and depotphoresis disinfection group (n = 10). As shown in Figure 1, each root was fixed in a 6-cm high round pot which was half-filled with 0.9% sterile saline and had two holes in its lid, one to place the root, and the other to place the lip clip of Depotphorese®-system Original II.

In the depotphoresis group, root canals were rinsed with 10 ml of sterile saline. According to the manufacturer's instructions of Depotphorese®-system Original II, one part of the cupral was mixed with nine parts of calcium hydroxide-dispersed, and 2 mm^3 of the resultant mixture was placed in the coronal third of the root canal by means of a Heidemann Spatula and a plugger.[11] The depotphoresis electrode was inserted in the coronal third of the root canal to the full length of the electrode (5 mm), and the lip clip was inserted in the pot. The electric current was established and gradually increased to 1.5 mA/min. Three times with intervals of 5 min, and each time,

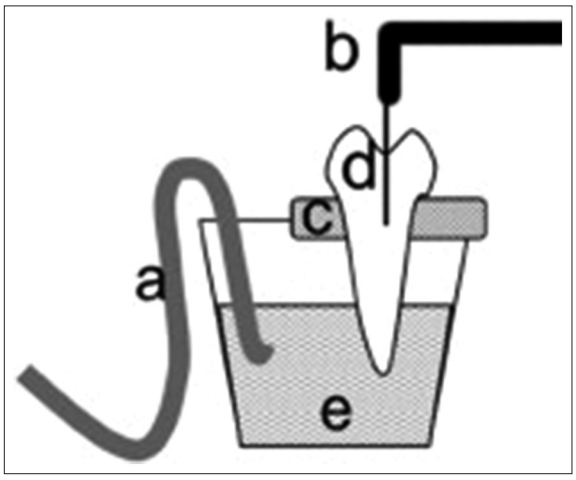

Figure 1: The experimental model (a) lip clip; (b) electrode; (c) lid; (d) tooth; (e) normal saline.

1.5 mA of electric current was established. Overall, 4.5 mA of electric current was transmitted. A final rinse with 10 ml sterile saline was performed.

In the NaOCl plus PUA group, irrigation with 20 ml of 5.25% NaOCl was done in 10 min, and the ultrasonic golden tip of Ultra X (Eighteeth, Changzhou, China) was used at low power within 1 mm of the apical foramen, and agitation of the irrigant was performed for 60 s. A final rinse with 10 ml sterile saline was done.

Each canal was then instrumented for 10 s with a sterile #30 K-file to obtain dentin shavings and sampled with two sterile #35 paper points which remained in the root canal for 1 min and then were moved to Eppendorf tubes containing 1 ml of TSB medium.

After vortexing the tubes for 10 s, 0.1 ml of the 1:10 dilutions was transferred to brain-heart infusion agar plates and incubated at 37°C for 48 h, and CFU per ml was counted.

Phase 2: The specimens with canal obstruction

The root canals were instrumented to F1 (ProTaper Universal; Dentsply Sirona, Ballaigues, Switzerland) to the apical foramen. Irrigation with 5.25% NaOCl was performed between each file insertion. A weak point was created on F3 rotary file (ProTaper Universal; Dentsply Sirona, Ballaigues, Switzerland) in the area between D3 and D4 by a round high-speed bur. The weakened file was rotated in the root canal at high torque (6 N/cm) to separate the file in the canal. A radiograph was taken to confirm that the separated file is located in the middle third of the canal. Next, the apical foramen was enlarged using Gates-Glidden bur #2 to a depth of 2 mm through the retrograde direction. To remove the smear layer, a final irrigation sequence was performed similarly to the group without obstruction. The roots were placed in Eppendorf tubes containing 2 ml of TSB medium and then autoclaved for 30 min at a pressure of 15 PSI and temperature of 121°C. Then, the tubes were incubated at 37°C for 24 h. A sterile micropipette was used to aspirate TSB and deliver 1 ml of *E. faecalis* suspension with a standard concentration of 0.5 McFarland (1.5×10^8 CFU/ml) into the root canal and around the root so as to immerse the root in the *E. faecalis* suspension. After that, the tubes were incubated at 37°C for 24 h, and bacterial viability was determined by CFU count. Subsequently, the specimens were incubated at 37°C for 21 days. To prohibit dehydration of the specimens during incubation, the TSB medium was replenished every 3 days.

The roots were randomly divided into NaOCl plus PUA group (n = 10) and depotphoresis disinfection group (n = 10).

The apical third of the specimens was sealed with wax, and also a hole was made in it so as to allow the electric current to flow. Root canals assigned to the depotphoresis group were treated exactly the same as the roots without obstruction which was described previously. In NaOCl plus PUA group, root canals were treated similar to the roots without obstruction except that the ultrasonic tip was placed in the coronal third of the root canal. Thereafter, the wax covering apical foramen was removed, and the canal was instrumented for 10 s with a sterile #30 K-file from a retrograde direction to obtain dentin shavings. Sampling was performed by two sterile #35 paper points which were described previously. Other procedures were the same as the group without canal obstruction, and CFU/ml was counted.

Statistical analysis

The CFU mean of the two samples of each specimen was calculated, and a log transformation of the CFU count was done to normalize the data before statistical analysis. Data were analyzed statistically using the Kruskal–Wallis test, with a significance level at $P < 0.05$, to indicate differences between depotphoresis and NaOCl plus PUA groups.

RESULTS

Phase 1

One of 10 depotphoresis specimens and four of 10 NaOCl plus PUA specimens showed no bacterial growth in any of their two samples. The means and standard deviations of each group are reported in Table 1. Using the Kruskal–Wallis test, the Log CFU of the depotphoresis group was significantly lower than NaOCl plus PUA group (P = 0.006).

Phase 2

The bacterial growth of two of the 10 specimens in the NaOCl plus PUA group was negative. The means and standard deviations of each group are reported in Table 2. Using the Kruskal–Wallis test, the Log CFU of the depotphoresis group was significantly lower than the NaOCl plus PUA group (P = 0.009).

Table 1: Mean, standard deviation, lowest, highest, and median values for log colony-forming units of the specimens without canal obstruction

Group	Number of specimens	Number of specimens with positive culture	Mean	SD	Least	Most	Median
Depotphoresis	10	9	1.15	2.08	−0.30	6.40	0.40
NaOCl plus PUA	10	6	5.47	3.54	0.40	8.74	6.70

NaOCl: Sodium hypochlorite; PUA: Passive ultrasonic agitation; SD: Standard deviation

Table 2: Mean, standard deviation, lowest, highest, and median values for log colony-forming units of the specimens with canal obstruction

Group	Number of specimens	Number of specimens with positive culture	Mean	SD	Least	Most	Median
Depotphoresis	10	10	2.34	0.99	0.81	3.24	2.84
NaOCl plus PUA	10	8	6.30	2.11	1.94	8.30	6.98

NaOCl: Sodium hypochlorite; PUA: Passive ultrasonic agitation; SD: Standard deviation

DISCUSSION

In either phase of the current study, depotphoresis treatment had a significantly greater reduction in Log CFU of *E. faecalis* than NaOCl plus PUA treatment.

Owing to the high prevalence of *E. faecalis* in persistent endodontic infections,[12-14] its single-species biofilm was used in this study. The difficulty of *E. faecalis* biofilm eradication is supported by the studies that have shown its ability to invade dentinal tubules[15] and form biofilms in medicated root canals.[16] Benefits of monospecies biofilm model systems are simplicity, standardization, and control,[17] although they are more susceptible to antimicrobial treatment than multispecies biofilms.[18-21]

According to similar studies,[22,23] standardization of the specimens was done by shaping the root canals 0.5 beyond the apical foramen. Removal of the smear layer was performed to get the maximum penetration of *E. faecalis* biofilm. Because there were no similar studies for the second phase of the present study, it seems, the best possible method that could simulate the clinical situation was adopted to allow sampling from the apical portion of the obstructed canal.

A low current electric field of about 5 mA was used to mobilize ions derived from cupral paste according to Fuss *et al*.'s study,[6] which eliminated all bacteria to a depth of 500 μm in dentinal tubules. Lin *et al*. applied a current of 10 mA that was more effective than pure calcium hydroxide in depths of 200–500 μm.[10] One study used a current of 15 mA that was the most effective against Gram-positive cocci.[24] The manufacturer's instructions of Depotphorese®-systems Original II recommended 5 mA in each session of treatment.[11]

It has been demonstrated that the maturity of the biofilm increases its resistance to NaOCl.[20,25] Because *E. faecalis* biofilm gets mature in 14–21 days,[26] a 3-week incubation was considered in the majority of studies.[17]

Zou *et al*. noted that NaOCl penetration depth in the dentinal tubules was 77–300 μm.[27] Haapasalo and Orstavik reported that after 3 weeks, *E. faecalis* caused dense infection of dentinal tubules to a depth of 400 μm, and the front of infection could reach 800–1000 μm.[15] Furthermore, bacteria can reside in uninstrumented recesses of the main canal.[28] 6% NaOCl eradicated 99.99% of *E. faecalis* biofilm bacteria *in vitro*;[29] however, its anti-biofilm efficacy was lower *in vivo*.[28,30] In the present study, 5.25% NaOCl plus PUA could not make all the root canals free of bacteria which could be related to NaOCl penetration depth, inaccessible areas of the canal, and decreased NaOCl anti-bacterial efficacy in the root canal.

Laser activation of irrigation has been shown to increase NaOCl penetration in dentinal tubules,[31] and NaOCl irrigation plus photon-induced photoacoustic streaming was more effective than NaOCl irrigation alone in the eradication of *E. faecalis* biofilm.[32] However, iontophoresis using Cu was more effective than laser therapy against both Gram-positive and Gram-negative bacteria.[24]

It has been claimed by the manufacturer that inaccessible areas of the root canal can be disinfected by depotphoresis.[11] Owing to fractured file conductivity, an electric current can be established in the apical portion of the canal, and the presence of the copper, calcium, and hydroxide ions can lead to disinfection. The quantity of the copper ion can be determined by spectrophotometry, which can be conducted in future studies.[33]

Intracanal sampling technique was one of the limitations of this study as passive sampling may not detect the bacteria that reside in inaccessible areas of the root canal which can cause false-negative results.[34] Future *in vitro* and *in vivo* studies can be conducted using newer methods of spectrophotometry like microspectroscopy,[35] and confocal laser scanning microscopy can be used in future *in vitro* studies.

CONCLUSION

Treatment with depotphoresis was significantly more effective than NaOCl irrigation plus PUA treatment in either teeth with canal obstruction or teeth without canal obstruction.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.
2. Möller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475-84.
3. Nair PN, Sjögren U, Krey G, Kahnberg KE, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: A long-term light and electron microscopic follow-up study. J Endod 1990;16:580-8.
4. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.
5. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
6. Fuss Z, Mizrahi A, Lin S, Cherniak O, Weiss EI. A laboratory study of the effect of calcium hydroxide mixed with iodine or electrophoretically activated copper on bacterial viability in dentinal tubules. Int Endod J 2002;35:522-6.
7. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: A systematic review and meta-analysis. Int Endod J 2007;40:2-10.
8. Böttcher DE, Hirai VH, Da Silva Neto UX, Grecca FS. Effect of calcium hydroxide dressing on the long-term sealing ability of two different endodontic sealers: An *in vitro* study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;110:386-9.
9. Knappwost A. The depotophoresis procedure with Copper-calcium hydroxide used for systematic healing. A leading alternative in endodontics. German Dental Journal 1993;102:618-28.
10. Lin S, Tsesis I, Zukerman O, Weiss EI, Fuss Z. Effect of electrophoretically activated calcium hydroxide on bacterial viability in dentinal tubules – *In vitro*. Dent Traumatol 2005;21:42-5.
11. Humanchemie GmbH. Depotphorese® system Original II Instructions. Depotphorese® System Original II. Available from: https://www.manualslib.de/manual/725776/Humanchemie-Depotphorese-Original-Ii.html. [Last accessed on 2023 Jun 10].
12. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1-7.
13. Rôças IN, Hülsmann M, Siqueira JF Jr. Microorganisms in root canal-treated teeth from a German population. J Endod 2008;34:926-31.
14. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J 2003;36:1-11.
15. Haapasalo M, Orstavik D. *In vitro* infection and disinfection of dentinal tubules. In: Journal of Dental Research. Duke St, Alexandria, VA 22314: American Associations Dental Research 1619; 1988. p. 760.
16. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93.
17. Swimberghe RC, Coenye T, De Moor RJ, Meire MA. Biofilm model systems for root canal disinfection: A literature review. Int Endod J 2019;52:604-28.
18. Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol 2006;72:3916-23.
19. Kara D, Luppens SB, Cate JM. Differences between single- and dual-species biofilms of *Streptococcus mutans* and *Veillonella parvula* in growth, acidogenicity and susceptibility to chlorhexidine. Eur J Oral Sci 2006;114:58-63.
20. Ozok AR, Wu MK, Luppens SB, Wesselink PR. Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus* (micromonas) micros. J Endod 2007;33:819-22.
21. Schwering M, Song J, Louie M, Turner RJ, Ceri H. Multi-species biofilms defined from drinking water microorganisms provide increased protection against chlorine disinfection. Biofouling 2013;29:917-28.
22. Siqueira JF Jr., Machado AG, Silveira RM, Lopes HP, de Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination

of *Enterococcus faecalis* from the root canal, *in vitro*. Int Endod J 1997;30:279-82.
23. Mozayeni MA, Haeri A, Dianat O, Jafari AR. Antimicrobial effects of four intracanal medicaments on *Enterococcus faecalis*: An *in vitro* study. Iran Endod J 2014;9:195-8.
24. Gergova RT, Gueorgieva T, Dencheva-Garova MS, Krasteva-Panova AZ, Kalchinov V, Mitov I, *et al.* Antimicrobial activity of different disinfection methods against biofilms in root canals. J Investig Clin Dent 2016;7:254-62.
25. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 2012;38:1376-9.
26. Guerreiro-Tanomaru JM, de Faria NB Jr., Duarte MA, Ordinola-Zapata R, Graeff MS, Tanomaru-Filho M. Comparative analysis of *Enterococcus faecalis* biofilm formation on different substrates. J Endod 2013;39:346-50.
27. Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod 2010;36:793-6.
28. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:231-52.
29. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. J Endod 2006;32:527-31.
30. Onçağ O, Hoşgör M, Hilmioğlu S, Zekioğlu O, Eronat C, Burhanoğlu D. Comparison of antibacterial and toxic effects of various root canal irrigants. Int Endod J 2003;36:423-32.
31. Akcay M, Arslan H, Mese M, Durmus N, Capar ID. Effect of photon-initiated photoacoustic streaming, passive ultrasonic, and sonic irrigation techniques on dentinal tubule penetration of irrigation solution: A confocal microscopic study. Clin Oral Investig 2017;21:2205-12.
32. Al Shahrani M, DiVito E, Hughes CV, Nathanson D, Huang GT. Enhanced removal of *Enterococcus faecalis* biofilms in the root canal using sodium hypochlorite plus photon-induced photoacoustic streaming: An *in vitro* study. Photomed Laser Surg 2014;32:260-6.
33. Gürkan R, Altunay N. A reliable method of quantification of trace copper in beverages with and without alcohol by spectrophotometry after cloud point extraction. Quim Nova 2013;36:1146-54.
34. Sathorn C, Parashos P, Messer HH. How useful is root canal culturing in predicting treatment outcome? J Endod 2007;33:220-5.
35. Herzog DB, Hosny NA, Niazi SA, Koller G, Cook RJ, Foschi F, *et al.* Rapid bacterial detection during endodontic treatment. J Dent Res 2017;96:626-32.