

Comparative antimicrobial efficacy of 2% chlorhexidine and Cupral paste along with electrophoresis in the apical region of root canals obstructed with a fractured instrument

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Abstract

Background and Objectives: Fracture of rotary files in the root canal system is a relatively common incident that obstructs access to the apical region and negatively affects treatment outcome. This study aimed to compare the antimicrobial efficacy of 2% chlorhexidine (CHX) and Cupral paste along with electrophoresis in the apical region of root canals obstructed with a fractured instrument.

Materials and Methods: In this ex-vivo experimental study, the root canals of 72 extracted single-rooted single-canal human teeth were instrumented, sterilized, and inoculated with *Enterococcus faecalis* (*E. faecalis*). A rotary file was intentionally fractured at 3 mm from the apex. The teeth were randomly divided into 6 groups of positive control, electrophoresis with 2% CHX, electrophoresis with Cupral paste, electrophoresis alone, irrigation with 2% CHX alone, and application of Cupral paste alone. The apical 3 mm of the root canals was then cut, and the number of colony-forming units (CFUs) in this region was counted. Data were analyzed by one-way ANOVA and Tukey's test.

Results: All interventions decreased the bacterial count in the apical region. The difference in the bacterial count was significant among the 6 groups ($P=0.00$). Electrophoresis with Cupral paste showed superior antimicrobial efficacy than other groups, but its efficacy was not significantly higher than that of electrophoresis with 2% CHX ($P>0.05$).

Conclusion: Electrophoresis with 2% CHX and Cupral paste yielded comparably efficacy for reduction of *E. faecalis* count in the apical region of root canals obstructed by a fractured instrument.

Keywords: Electrophoresis; Chlorhexidine; Cupral Paste; fractured instrument

Introduction

Procedural errors commonly occur during endodontic treatment. Instrument fracture in the root canal system is a common procedural error that can compromise the outcome of endodontic treatment if not properly managed [1,2]. Endodontic files are often made of stainless steel or nickel-titanium (NiTi) [3] and may fracture in the root canal system due to misuse or excessive use [4]. Instrument fracture often occurs in the apical region of root canals [5-8]. The novel NiTi rotary

files have a higher risk of fracture [9]. The reported percentage for fracture of rotary NiTi files ranges from 0.4% to 4.6% [10-12]. Fracture of NiTi files often occurs unexpectedly, and new files are no exception [13-17]. Deformation of NiTi files cannot often be detected without magnification; while, fracture of stainless steel files is more predictable due to visible deformation of the file [18-20]. Not retrieving the fractured instrument from the root canal system often decreases the success rate of treatment. Evidence shows a higher rate of treatment

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failure by 19% in the presence of a fractured instrument in the root canal system [21]. Therefore, removing the fractured instrument from the root canal system is critical to maximize treatment success. The prognosis of teeth with fractured instrument depends on the level of cleaning and shaping of the canal when a fracture occurs, pulp status, periradicular tissue status, and success/failure of bypassing or removing of the fractured instrument [22].

Several strategies have been proposed to manage fractured instrument, such as maintaining the fractured piece in the root canal system and obturation of the rest of the root canal space, bypassing the file and accessing the apical end, and using different kits available for retrieval of fractured instruments. However, in many cases, removal or bypassing the fractured instrument is difficult, time-consuming, and costly and may be associated with some inevitable side effects and complications such as weakening of the root structure, ledge formation, or root perforation [23]. Endodontic treatment failure occurs due to the activity of residual microorganisms in the root canal system [24]. In non-vital teeth, oral microorganisms colonize the pulp chamber and root canal space and form a microbial biofilm, making them resistant to disinfecting agents and intracanal medicaments [25]. The causative microorganisms in secondary endodontic infections are often different from those responsible for primary endodontic infections. The microorganisms responsible for reinfection of the root canal system are more resistant and lead to refractory infections. *Enterococcus faecalis* (*E. faecalis*) is the main culprit responsible for endodontic reinfection [26]. In teeth with fractured instrument, microorganisms remaining in the obstructed apical part of the root are responsible for reinfection. This is especially true when the fractured instrument has obstructed the apical third of the root canal or an area apical to a severe curvature [27].

Ultrasonic instruments have also been suggested for the retrieval of fractured instrument. However, retrieving the fracture segment from the apical third of the root canal is almost impossible with ultrasonic instruments. Moreover, effective use of ultrasonic instruments requires coronal flaring of the root canal system, which weakens the root structure, and can lead to ledge formation or root perforation [28].

The use of electrophoresis along with Cupral paste (which is an antimicrobial paste composed of calcium hydroxide and copper ions) was first suggested in 1993 as an effective method for elimination of microorganisms from the apical third of very narrow root canals [29]. Further investigations later confirmed the acceptable clinical efficacy of this technique [30]. The efficacy of electrophoresis with Cupral paste for eliminating bacteria and its good penetration into dentinal tubules has been previously documented [31]. In addition, a recent study reported that although the root canal preparation increases the copper ion output in an ex-vivo environment, the amount of copper extruded from the root apex end was lower than the toxic dose [32]. Thus, this technique may be suitable for disinfection of the part of the root canal obstructed with a fractured instrument without its removal and the related

consequences. The aim of the study was to compare the antimicrobial efficacy of Cupral paste with electrophoresis in the root canals with a fractured instrument in the apical region.

Materials and Methods

This ex-vivo, study evaluated 72 human single-rooted single-canal teeth that had been extracted due to poor periodontal prognosis or as part of orthodontic treatment. The ethics committee of AJA University of Medical Sciences approved the study protocol (IR.AJAUMS.REC.1398.255). The inclusion criteria were single-canal, single-rooted teeth with closed apices, canal curvature < 25°, absence of calcification, internal resorption, and root caries. In addition, Mesiodistal and buccolingual periapical radiographs were obtained to ensure that the teeth met the inclusion criteria. The sample size was calculated assuming $\alpha=0.05$, $\beta=0.2$, and study power of 80%, and the teeth were selected using convenience sampling. To standardize the root length, all teeth were decoronated by a high-speed diamond disc such that the remaining root length was 15 mm in all teeth. Next, a #10 K-file was introduced into the canal until the file tip was visible at the apex to determine the working length. The file length was then measured, and 1 mm was subtracted from this length to determine the working length. Teeth in which a #10 K-file could not easily reach the apical foramen, those with an apical size larger than a #20 K-file, and those without a round cross-section were excluded and replaced. The root canals were then instrumented with ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to F2 at a speed of 300 rpm and 1.5 Ncm torque. After instrumentation, the root canals were irrigated with 5.25% sodium hypochlorite using a 5 ml syringe with a 23-gauge needle. After root canal preparation, each tooth was packed and autoclave-sterilized at 121°C for 20 min. Next, 5 teeth were randomly selected for negative control and incubated in brain heart infusion (BHI) broth (Conda Laboratories, Madrid, Spain) at 37°C for 48 h.

E. faecalis (ATCC29212) was obtained from the Iranian Pasteur Institute in lyophilized form. BHI broth was added to it such that it completely covered the surface of the solid powder. It was then incubated at 37°C for 15 min to create a microbial suspension. The prepared microbial suspension was transferred to a solid culture medium by a sterile syringe. After bacterial culture on BHI agar plate (Conda Laboratories, Madrid, Spain) and 24 h of incubation, safranin staining was performed and the culture was inspected under a light microscope at x100 magnification to ensure the correct type of microorganism, and no contamination of culture medium. For bacterial culture in the liquid medium, one independently grown colony was removed from the plate by a sterile loop under aseptic conditions and transferred into a test tube containing 3 mL of BHI broth. The test tube was then incubated at 37°C for 24 h. In order to standardize the microbial suspension used for inoculation, sterile BHI broth was transferred to the test tube containing bacteria and the turbidity was adjusted at 0.5 McFarland standard concentration. The obtained standard mi-

crobial suspension was transferred into 72 test tubes under aseptic conditions such that each test tube contained 1 mL of the suspension. Next, each root was placed in one test tube with a sterile hemostat. The test tubes were incubated at 37°C for 2 days. Afterward, 1 mL of sterile BHI broth was added to each test tube by a sterile syringe under aseptic conditions. This process was repeated for one week. To ensure no contamination with other microorganisms during this period, a few drops of the contents of two randomly selected test tubes were collected by a sterile loop and Gram-stained.

The apical 3 mm of a ProTaper F2 rotary file (Dentsply Maillefer, Ballaigues, Switzerland) was cut by a diamond bur and high-speed hand-piece. Next, a small notch was created at 4 mm from the tip of the remaining file segment to weaken the file using a knife-edge bur. The files were then autoclave-sterilized at 121°C for 20 min. After 1 week of incubation, each tooth was removed from the test tube with a sterile hemostat under aseptic conditions. The sterilized file was introduced into the root canal and rotated until the apical 4 mm segment broke at the notch. To further aggravate the canal obstruction by the fractured file, a small amount of sterile dentin chips (collected by using a #4 Gates-Glidden drill in the canal of a sterile tooth) was added to each root canal. A radiograph was obtained from each tooth to ensure instrument fracture at the desired location (apical 3 mm). Afterward, the teeth were randomly divided into 6 groups (n=12) according to the type of intervention to be performed:

G1: 2% CHX + electrophoresis

G2: Cupral paste + electrophoresis

G3: Electrophoresis alone

G4: 2% CHX alone

G5: Cupral paste alone

G6: Positive control group

In electrophoresis groups (G1, G2 and G3), alginate (Chromogel, Iran) was used as a periodontal tissue substitute. For this purpose, alginate powder was mixed with water and transferred into 5 ml Eppendorf plastic microtubes. The roots were mounted in the tubes such that their coronal 2 mm was out of the alginate.

In G1, 2% CHX (Cerkamed, Gluco) was injected into the canal through the orifice by a sterile syringe such that it did not overflow after insertion of the cathode end of the depot phoresis (Humanchemie GmbH, Germany) in the root canal filled with CHX. Next, the tooth was mounted in a microtube containing alginate. The anode end of the device was placed in the alginate next to the root without contacting it. The electric current was then started (0.9-1.2 mA) and it was automatically discontinued when it reached 7.5 mA min. and incubated at 37°C under humid conditions.

In G2, a small amount of distilled water was added to Cupral paste (Humanchemie GmbH, Germany) to reach a creamy consistency. The paste was then delivered into the canal by a #20 hand file with counter-clockwise motion up to the orifice. The roots were then mounted in microtubes containing alginate. The rest of the procedure was the same as that in G1. In

G3, 20 µL of sterile saline was used in each root canal to enhance electric conduction. The rest of the procedure was the same as that in G1. In G4, 2% CHX was used alone. In G5, Cupral paste was used alone as in G2. In G6, no intervention was performed, and this group served as the positive control. The roots in all 6 groups were then removed from the microtubes and incubated at 37°C under humid conditions for 1 week. After 1 week of incubation, the interventions were repeated and the roots were incubated again at 37°C under humid conditions for 1 more week.

Finally, the antimicrobial efficacy of the interventions was evaluated by counting the colony-forming units (CFUs). For this purpose, the apical 3 mm of the roots was cut under aseptic conditions, and the root canal of the separated segment was filled with sterile BHI broth. A sterile #20 paper point was placed inside the canal with a sterile hemostat. After 1 min, the upper part of the paper point which was out of the canal was cut, and the remaining segment was transferred into a test tube containing 1 mL of sterile BHI broth with a sterile hemostat. The test tube was shaken (in order to separate the bacteria from the paper point), and it was then incubated at 37°C for 2 h. Next, 20 µL of the contents of each tube was transferred onto a culture plate containing BHI agar with a disposable insulin syringe under aseptic conditions. The plates were incubated at 37°C for 24 h. The number of CFUs was counted after incubation.

Table 1: pain status in the studied patients

Group	Days Post-operatively	mean standard± deviation	Min	Max
Intervention Group	3rd	1.12 ± 4.65	3	7
	7th	1.05± 1.19	0	3
Control Group	3rd	1.51 ± 3.15	0	6
	7th	0.26 ± 0.45	0	1

Data were analyzed using SPSS version 25 (SPSS Inc., IL, USA). The mean and standard deviation of colony count were reported for each group. One-way ANOVA was used for the general comparison of the groups, while the Tukey's test was applied for pairwise comparisons. A P<0.05 was considered statistically significant.

Results

No microbial growth in the 5 negative control tubes ensured the complete success of sterilization. Also, observing Gram-positive diplococci under a microscope ensured a culture of the correct type of microorganism, and no contamination with other microorganisms. **Table 1** presents the measures of central dispersion of colony count in the six groups. After ensuring normal distribution of data with the Kolmogorov-Smirnov test, one-way ANOVA was applied for general comparison, which revealed a significant difference in colony count between the groups (P=0.00). Subsequently, pairwise comparisons of the

groups regarding colony count were carried out by the Tukey's test (**Table 2**). The results showed that all interventions significantly decreased the *E. faecalis* colony count, except for electrophoresis alone. Electrophoresis with Cupral paste showed superior antimicrobial efficacy than other groups, but its efficacy was not significantly higher than that of electrophoresis with 2% CHX ($P>0.05$).

Table 2: Wound Healing Index in the studied patients

Group	Days Postoperatively	mean standard± deviation	Min	Max
Intervention Group	7th	0.59 ± 3.30	2.5	4.25
	14th	0.45 ± 3.84	3	4.5
Control Group	7th	0.54 ± 4.05	3	4.75
	14th	0.24 ± 4.57	4.25	5

Discussion

This study compared the antimicrobial efficacy of 2% CHX and Cupral paste along with electrophoresis in the apical region of root canals obstructed with a fractured instrument. Considering the complications of overuse of instruments in the root canal system, the use of intracanal medicaments and their activation with electric current is a conservative antimicrobial approach [33]. The high penetration power of the electric current can help the transport of ions with antimicrobial activity through the obstruction. The type of charged particles is an essential factor determining their antimicrobial activity. Previous studies have confirmed the optimal efficacy of electrophoresis along with Cupral paste for elimination of microorganisms from the root canals with difficult access [30,31,34]. Considering the greater antimicrobial effect of CHX than sodium hypochlorite and calcium hydroxide on many microorganisms particularly *E. faecalis*, as well as the fact that CHX contains charged cations, which can be directly influenced by the electric current in electrophoresis [32,35], CHX was compared with Cupral paste in this study. The results showed that all interventions caused a significant reduction in bacterial count in the obstructed part of the root canal. Cupral paste with electrophoresis was superior to other interventions. Electrophoresis without saline only caused no significant change in colony count compared with the control group. However, descriptive statistics revealed that electric current decreased the count of viable bacteria in the obstructed segment, which can be due to the dissolution of microorganisms in saline passing through the obstructed region. The charged ions of the medicament that reach the obstructed segment under the influence of the electric current are responsible for the elimination of biofilm; while the electric current alone cannot play a significant role in elimination of biofilm. The antimicrobial efficacy of Cupral paste plus electrophoresis was not significantly higher than that of 2% CHX alone, and 2% CHX plus electrophoresis. However, all groups showed a significant difference with the control group and electrophoresis alone, and Cupral paste plus electrophoresis was the most effective intervention.

Evidence shows that 2% CHX alone is significantly more effective than calcium hydroxide for the elimination of *E. faecalis*

[17]. Although the difference between CHX + electrophoresis, and Cupral paste + electrophoresis did not reach statistical significance in our study, descriptive statistics showed that Cupral paste + electrophoresis was slightly more effective for the elimination of bacteria, which can be due to the greater effect of electric current on copper ions present in Cupral paste. Considering the optimal antibacterial activity of copper [20], the obtained results can be due to the copper ions and other components of the Cupral paste reaching the obstructed part of the root canal. Cupral paste can degrade the microbial proteins and cause proteolysis. Also, it can remove the sulfur from the amino acids in the lipopolysaccharide membrane. Thus, Cupral paste is effective against both viable bacteria and spores. Although there are some concerns regarding the possible cytotoxicity of Cupral paste for the periapical tissues [12], Sachdeva et al. [36] demonstrated the positive effects of copper ions on periapical tissue healing.

Our study did not demonstrate the significant effect of the addition of electrophoresis to the use of CHX and Cupral paste for elimination of *E. faecalis*. However, their combination with electrophoresis showed slightly higher antimicrobial activity due to the effect of electric current. Previous studies have also confirmed the optimal effect of electric current on the penetration depth of intracanal medicaments [30,37].

Search of the literature by the authors yielded no similar study on this topic to compare our results with, which was a limitation. Also, this study had an in vitro design, which limits the generalization of results to the clinical setting. Moreover, only one type of microorganism was evaluated in this study. Future studies should evaluate other types of microorganisms responsible for endodontic infections. Absence of a significant difference between 2% CHX and Cupral paste with/without electrophoresis may be due to our small sample size. Thus, future studies with a larger sample size are required to find more accurate results. Also, the efficacy of 1% silver nitrate as an intracanal medicament along with electrophoresis should be investigated and compared with Cupral paste in future studies. Studies on the antimicrobial efficacy of the tested interventions at different depths of dentinal tubules are also recommended.

Conclusion

Activation of 2% CHX and Cupral paste with electrophoresis yielded comparably optimal efficacy for reduction of *E. faecalis* count in the obstructed segment of the root canal system. Despite the slightly higher efficacy of Cupral paste + electrophoresis, 2% CHX + electrophoresis can be used as an alternative antimicrobial approach due to the existing concerns regarding the cytotoxicity of copper ions.

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