Original Article

Comparing antimicrobial effect of cupral and calcium hydroxide paste with electrophoresis in apical part of root canals blocked by separated instrument

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Abstract

Introduction: This study compared the antimicrobial effect of electrophoresis with calcium hydroxide and electrophoresis with cupral paste in the apical area blocked by the separated instrument.

Materials and Methods: A total of 72 single-rooted human teeth were involved in the study, each decoronated to a length of 15 mm. Following the determination of the working length and root canal preparation, the teeth were autoclaved and subsequently infected with *Enterococcus faecalis*. Intentional fracture of a rotary instrument occurred 3mm above the apical terminus of the canal. The samples were then randomly assigned to six groups: Groups G1 and G2 received Cupral paste (Humanchemie GmbH, Germany) and calcium hydroxide paste (Humanchemie GmbH, Germany), respectively, with the application of electric current. In G3, sterile normal saline was used with electrophoresis. For G4 and G5, Cupral and calcium hydroxide pastes were applied to the root canal without electrical current, respectively. G6 was used as the positive control. Following all interventions, the 3mm apical segment of the specimens was removed, and the antimicrobial effect was assessed by counting colony-forming units (CFUs) in this canal area.

Results: Cupral paste, Cupral paste with electrophoresis, and calcium hydroxide with electrophoresis demonstrated a significant reduction in bacteria beyond the separated instrument ($p<0.05$). However, calcium hydroxide and electrophoresis alone did not exhibit statistically significant antimicrobial activity ($p> 0.05$). There was no statistical difference observed between Cupral paste activated by electrophoresis and calcium hydroxide activated by electrophoresis ($p> 0.05$).

Conclusion: Electrophoresis enhanced medicament penetration and antimicrobial efficiency in the canal blocked by the separated instrument. Cupral paste activated by electrophoresis demonstrated superior performance in all interventions.

Key Words: Calcium hydroxide; Electrophoresis; *Enterococcus faecalis*

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Introduction

The rising utilization of nickel-titanium rotary files has led to a notable increase in file fractures during root canal treatments and clinical investigations have indicated its prevalence ranging from 0.39% to 5% (1). Conventional management of broken instruments include attempts to remove, bypass or leaving the broken instruments in situ (2). Due to technological advances such as the widespread use of dental operating microscopes and ultrasonic devices, removal of broken instrument from the canal has been associated with higher success rates and fewer complications (3). Fractured instruments located in apical third of curved canals are often considered impossible to remove because of poor visibility and accessibility (4). In cases where retrieval is not possible, bypassing the fractured instrument allows for the complete cleaning, shaping, and obturation of the entire root canal space, even if the remaining fragment is situated in the middle or apical third of curved canals (5, 6). Herein, there might be an elevated risk of creating an artificial canal, secondary instrument separation, ledge formation, and possible fragment extrusion beyond the apical foramen (7). Leaving the fragment in situ, is considered applicable when the fragment is in the apical third, beyond the root canal curvature (8). However, this method will be in line with compromised mechanical and chemical cleansing and shaping of the root canal space (9).

Presence of a separated instrument within the root canal space does not necessarily result in postoperative disease. Nevertheless, the presence of residual microbial infection affects the treatment prognosis (10). In such instances, the optimal strategy appears to involve eradicating pathogenic microorganisms beyond the obstructed region. This can be accomplished by introducing antimicrobial agents into the targeted area. Knappwost has reported a method for disinfection of root canals by using electrophoretically activated calcium hydroxide(CH) with copper(11). Cupral is a combination of CH and copper (Humanchemie, Germany), which was introduced by Adolf Knappvost (11). It has a stable balanced system and contains negatively charged copper hydroxide II nanoparticles and hydroxy coppurate anions, with high antimicorbial activity. It is effective against all aerobic and anaerobic microoganisms. Cupral is capable of eliminating the resistance of microflora and has an antimicrobial activity 100 times stronger than that of CH (12). Silver is also used to improve the properties of CH (12). Evidence shows that addition of copper and silver to CH can increase its antimicrobial activity (12, 13). In subsequent research, this method proved to be clinically acceptable (12).

The activation through electrophoresis resulted in a substantial enhancement of the antibacterial activity of CH or copper-infused calcium hydroxide (CH with copper) and their penetration into the dentinal tubules. (12, 14).

As far as the authors are aware, no previous study has assessed the antimicrobial impact of electrophoresis using CH and electrophoresis with Cupral paste in the apical region obstructed by a separated instrument. Therefore, the objective of the current study was to compare the antimicrobial effects of electrophoresis with CH and Cupral paste in the apical area obstructed by a separated rotary file.

Materials and Methods

Sample selection

The study protocol was approved by the Ethics Committee of AJAU (IR.AJAUMS.REC 1398.108). In this experimental *in vitro* study, a total of 72 single-rooted human teeth extracted for periodontal reasons were involved. Radiographs in both mesiodistal and buccolingual directions were taken for all teeth to confirm the presence of a single-canal internal anatomy, apical closure, a curvature angle of less than 25 degrees, and the absence of any calcification or internal resorption.

Root canal preparation

The teeth were decoronated resulting in a uniform length of 15 mm for all teeth. To determine the working length, a #10 K-file (Dentsply Maillefer) was inserted into the canals and moved until the tip of the file could be seen through the apical foramen and the

length measurement was recorded. Then, the working length was calculated as 1 mm shorter than the measured length. Specimens that faced difficulty reaching the apical foramen or had a size exceeding #20 in this region, as well as teeth lacking a rounded cross-section, were excluded from the study and substituted with alternative specimens. Root canal preparation was performed with Universal ProTaper System up to size #F2 (Ballaigues, Switzerland Maillefer, Dentsply) according to the manufacturer's recommended protocol. Recapitulation was performed between use of each file and root canals were irrigated with 5.25% sodium hypochlorite. To complete sterilization of the root canals, the samples were autoclaved at 121° C for 30 min. Five samples were randomly selected and each were individually incubated in a plate containing brain heart infusion (BHI) agar (Laboratorios Conda, Madrid, Spain) at 37 ° C for 48 h.

Contamination of samples

The standard bacterium *Enterococcus faecalis* (ATCC: 29212) was obtained from the Iranian Microbial Collection Center (Pasteur Institute). Afterwards, 72 specimens were placed in tubes containing 1 mL of microbial suspension with OD of 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Specimen were incubated at 37 ° C for 7 days. After 2 days, 1 mL sterile BHI broth was added to each tube. The procedure was done every 2 days for 1 week to ensure viability of the bacteria (15). Subsequently, a sterile #F2 rotary file (ProTaper System; Ballaigues, Switzerland, Maillefer, Dentsply) was notched with a diamond bur at its apical 3 mm to facilitate separation using a high-speed knife diamond bur (DIA-Swiss TEC, Switzerland).

After 7 days of incubation, the rotary file was broken 3mm coronal to the apical end of each canal (Figure 1). Then, a small amount of sterile dentine was poured into the canal to increase obstruction. Finally, a radiograph was taken to ensure that the file was broken in an ideal location.

Experimental protocol

The specimens were randomly divided into 6 groups. In group G1, the Cupral paste (Humanchemie GmbH, Germany) was inserted into the canal and the specimens was embedded in a microtube containing alginate as a conductive environment. The method was performed using an electrode inserted into the root canal space and another electrode was located outside the specimen according to Knappwost et al (11). To simulate clinical conditions, all specimens were incubated under humid condition for 1 week at 37, then the electrode of Depotphores (Humanchemie GmbH, Germany) was inserted into the root canal. Another electrode located outside the specimen. Electrical current was maintained in the 0.9-1.2mA range. Upon reaching the sum of the electrical current equal to 7.5mA/min, the device automatically cut off the current. In G2, CH paste (Humanchemie GmbH, Germany) was used and sterile normal saline was used in G3.

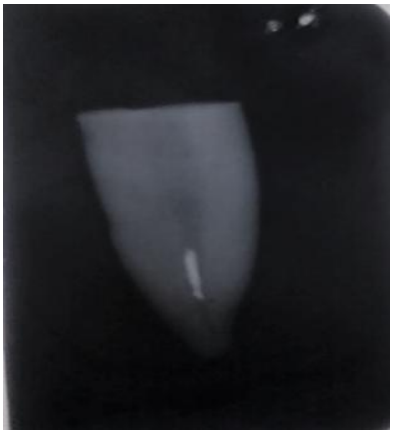

Figure 1. Radiographic image of separated instrument in the root canal

A 20 µl volume of sterile normal saline was introduced into the root canal to enhance the transmission of electrical current. Once inserted into the tube containing a conductive medium, the electrical current was reinstated, following a procedure similar to that of the preceding groups. For specimens in G4 and G5, Cupral and CH, respectively, were introduced into the root canal without the application of electrical current. Table 1 shows the experimental procedure performed in each group.

Table 1. Procedure performed in each group

Group	Intervention	Number
G1	Cupral with electrophoresis	12
G2	CH with electrophoresis	12
G3	Electrophoresis alone	12
G4	Cupral without electrophoresis	12
G5	CH without electrophoresis	12
G6	Positive control group	12

All specimens were incubated in a humid environment at a temperature of 37°C for a duration of 7 days to replicate clinical conditions. The prior procedures were replicated by introducing freshly prepared paste into the root canal. The apical 3mm of the specimens was cut, and the apical root canal was filled with sterile BHI broth, under aseptic conditions. Paper points were immediately placed into the root canal space to absorb the broth. Following a duration of 1 minute, the outer section of the paper was removed, and the remaining segment within the canal was transferred to 1mL of BHI broth. The tube was vortexed to release the microbes into the broth medium following incubation for 1 hour. A volume of 20 µL from the vortexed broth was subsequently introduced onto agar plates that were infused with BHI agar. The formation of bacterial colonies was evaluated after 24 h and the colony forming units (CFUs) were counted.

Statistical Analysis

One-way analysis of variance (ANOVA) test was used to compare the CFU means. *Post hoc* tests (Tukey's HSD) were performed to assess differences in the effects of different disinfection methods. The level of significance was set at $p<0.05$. All statistical analyses were performed with SPSS software (SPSS version 25, SPSS, Chicago, IL, USA).

Results

Diagram 1 shows the mean proliferation of bacterial colonies at different groups. According to ANOVA test, the difference between groups was significant in colony count ($P <0.001$). table 2 describes of the data related to CFUs.

A significant statistical decrease in the number of bacterial colonies was observed in Cupral, Cupral with electrophoresis, and CH with electrophoresis, in comparison to the control group ($p< 0.05$). However, CH and electrophoresis alone did not show statistically significant antimicrobial activity.

No statistical difference was found between CH, Cupral paste and CH activated by electrophoresis ($p> 0.05$). Cupral paste with electrophoresis showed a higher antimicrobial efficacy as compared with CH ($p< 0.05$), but this difference with Cupral paste and CH with electrophoresis was not significant ($p> 0.05$). (See table 3).

Discussion

The primary objective of the current study was to assess and compare the antimicrobial efficacy of Cupral paste with electrophoresis and CH with electrophoresis in the apical third region obstructed by a separated instrument. The most favorable outcomes were achieved by incorporating electric current into the intracanal medicament. This outcome aligns with the findings of previous studies, supporting the utility of this method in challenging and difficult-to-access root canals (16, 17).

The application of electrophoresis alone exhibited minimal impact on bacterial biofilm. Hence, it appeared that the primary reason for biofilm destruction lied in the charged components of the medication within the canal, which penetrated beyond the obstructed area under the influence of electric current. Electric current itself did not seem to play a significant role in the destruction of microbial biofilm. Creating an electrical current without intracanal medicaments reduced the living bacteria behind the separated file but did not make any significant difference with the control group. It was also shown that electrophoresis allowed greater access of polar therapeutic molecules to deep skin areas by 10-2000 times compared with conventional application of a medicament

Table 2. Description of the data related to CFU

Colony count												
Control		Electrophoresis		calcium		Cupral		Electro+Ca		Electro+Cup		P value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
9.3905	0.56280	8.9458	0.64936	7.9171	0.73474	7.4236	1.64317	8.4477	1.48941	4.2416	0.54934	<001

Table 3. Difference in microbial colony count in different groups

(I) Groups	(J) Groups	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Electrophoresis	.44474	.295	-.3990	1.2885
	calcium	1.47347*	.002	.5886	2.3584
	Cupral	1.96698*	<001	1.0557	2.8783
	Electro+Ca[1]	.94281	.050	-<0015	1.8861
	Electro+Cup[2]	5.14891*	<001	3.8149	6.4829
Electrophoresis	calcium	1.02873*	.024	.1438	1.9136
	Cupral	1.52225*	.002	.6109	2.4336
	Electro+Ca	.49807	.294	-.4452	1.4414
	Electro+Cup	4.70418*	<001	3.3701	6.0382
CH	Cupral	.49352	.301	-.4561	1.4431
	Electro+Ca	-.53066	.282	-1.5110	.4497
	Electro+Cup	3.67545*	<001	2.3150	5.0359
Cupral	Electro+Ca	-1.02418*	.046	-2.0284	-.0200
	Electro+Cup	3.18193*	<001	1.8041	4.5597
Electro+Ca	Electro+Cup	4.20611*	<001	2.8070	5.6053

1 Cupral with electrophoresis

2 CHs with electrophoresis

*P<0.05

(18). Based on the findings of the present study, employing CH as an intracanal medicament in instances of a separated instrument has the potential to decrease presence of bacterial beyond the area of separation. Additionally it was noticed that, copper paste worked in a similar manner compared with CH. Although the mean of grown colonies was lower in copper-medicated samples, statistical analysis of the findings did not prove any significant superiority of either substance over the other.

Use of CH in combination with electrophoresis had a significant effect on biofilms located apical to the separated instrument. However, according to the results of statistical analysis, this method did not have a significant advantage over the use of CH without electrophoresis and therefore is not in line with the results of Lin *et al.*(19) .

As per the present research, the effectiveness of copper paste intervention through electrophoresis was notably superior to electrophoresis alone and CH alone ($p < 0.05$). However, it did not exhibit this superiority over CH with electrophoresis and Cupral paste alone. However, the intervention of cupral paste with electrophoresis had a much better antimicrobial effect than other interventions (Diagram 1).

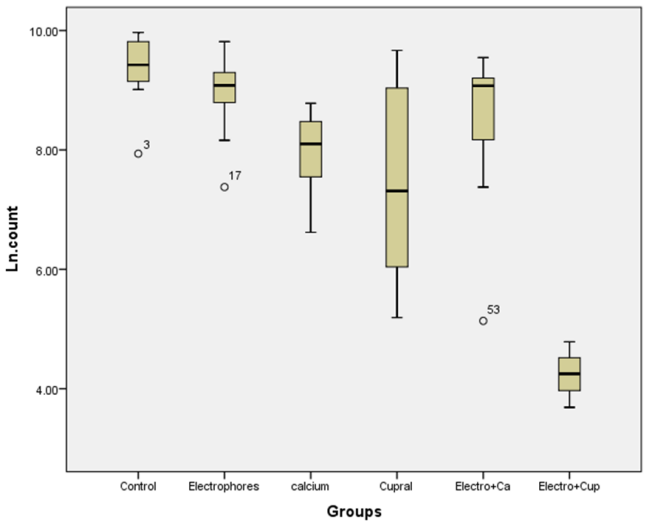

Diagram 1. The mean proliferation of bacterial colonies in the groups

Due to the remarkable antibacterial properties of copper (20), the penetration of copper ions and other components of copper paste beyond the separated instrument into canal can justify these results. Copper possesses the capability to dismantle microbial proteins through proteolysis and degrade lipopolysaccharide membranes by extracting sulfur from amino acids. Copper not only exhibits antibacterial effects on various bacterial growth forms but also demonstrates the capability to influence their spores. The difference between the mean colonies grown in the groups in which cupral was part of the intervention and in the groups in which CH was used confirms copper's high ability to eradicate bacteria. There was a possibility of toxicity of this substance on periapical tissues(13), but the study by Sachdeva *et al.* showed promising effects of copper ions on the repair of periapical tissues (21). Yousefshahi *et al.* reported that Cupral paste had higher antimicrobial activity than CH, which was in agreement with the results obtained from the current investigation (22).

Both copper paste and CH showed superior antimicrobial effect by electrophoresis and this difference showed a significant effect of electric current in the mentioned interventions. According to previous studies, penetration of the charged components of the drug into the canal inside the dentinal tubules could be increased under the influence of electric current. Penetration of these materials from the distance of the remaining file inside the canal and the inner wall of the canal can be much easier than penetration into dentin tubules. In addition, the cross section of the separated instrument inside the canal, the shape and size of flutes, as well as the amount of smear layer and dentin fragments in the blocked area can affect the penetration of antimicrobial components (19).

In the present study, maximum obstruction was created in the area by methods such as trying to lock the file inside the canal during separation and also using dentin fragments. Yet, the study results provided proof of the extensive infiltration of the medication into the canal beyond the separated instrument. The reduced count of viable bacteria following the application of Cupral paste with electrophoresis and CH with electrophoresis indicated a more substantial penetration of the components of these two substances under the impact of electric current.

Conclusion

The activation of Cupral and CH through electrophoresis improved their ability to eliminate Enterococcus faecalis in the canal obstructed by a separated instrument. The distinctive nature of Cupral paste particles contributes to its superior antibacterial activity under comparable conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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