

Measurement of Copper Ion Extrusion from the Apex of Human Teeth with Single Canal Following Electrophoresis

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ABSTRACT

Objective: Preventing bacterial overgrowth is an essential component of successful root canal treatment. Increasing the penetration of antimicrobial substances into the canal through electrophoresis is one of the possible methods. This study aimed to measure the copper ions extruding from the root apex after using an electrophoresis device (Depotphorese®) in human teeth with single canals.

Methods: In this *ex-vivo* study, thirty extracted human teeth with single canals were randomly divided into 2 groups. Group 1 as root canal preparation (WP) and group 2 as control (without root canal preparation). Each sample was individually fixed by alginate in a 5 ml sterile Eppendorf tube (microcentrifuge tube), and the root end was in contact with the distilled water solution. Ions derived from calcium hydroxide plus copper paste (Cupral®) which mobilized via a low current electric field (1.5 mA/min for 10 min) by Depotphorese® device. The copper ions in water solution were measured by spectrophotometric and supernatant methods.

Results: Copper ions were extruded from the end of the apex in two groups. However, based on the results of the t-test; copper ion penetration was significantly lower in the control group compared to the WP group ($P < 0.05$).

Conclusion: Electrophoresis increased copper extrusion from the apical foramen. 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. It seems that electrophoresis of copper plus calcium hydroxide mixture in endodontic treatments can be a safe procedure.

Keywords: Cupral, electrophoresis, preparation, root canal

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HIGHLIGHTS

- Cupral® efficiently counteracts endodontic infections. Yet, the mechanism of action have not been investigated.
- The preparation of the root canal increases the copper ion output in an *ex-vivo* environment.
- The amount of copper removed from the apex was not within the toxic range for periapical cells.

INTRODUCTION

The presence of the microorganisms and their byproducts within the root canal system is the main cause of the pulpal and periapical disease (1). One of the main objectives to achieve during endodontic therapy is reducing the number of microorganisms in infected root canals (2, 3). Thus, the elimination of the necrotic tissue and infected debris by irrigation and instru-

mentation is imperative (2, 4). Because of the mechanical cleansing limitation, it is essential to use intracanal medicament to eliminate bacteria in the root canal system (5). Several substances have been used to clean the root canal system.

Cupral® (HUMANCHEMIE, Alfeld, Germany) or a mixture of calcium hydroxide and copper has acceptable antimicrobial activity. Cupral contains highly dispersed calcium hydroxide $[Ca(OH)_2]$, copper sulfate (II) $(CuSO_4)$, calcium sulfate $(CaSO_4)$, copper hydroxide (II) $[Cu(OH)_2]$, methylcellulose $[C_6H_7O_2(OH) \times (OCH_3)_6]$, and distilled water (6).

It is reported that the application of the copper-calcium hydroxide ions under the influence of electric current could penetrate dentinal tubules (up to a depth of 0.5 mm in 7 days) and eliminate intracanal bacteria (7). Electrophoresis with Cupral® is a minimally invasive alternative

in endodontics. The success of this method is based on the effect of Cupral®. It is transported by an electrical field out of a small depot in the root canal orifice into all apical delta arms. This weak electrical field is related only to the oral cavity of the patient [8]. Indications for the use of electrophoresis with Cupral® in dentistry include the elimination of infectious processes in root canals, including roots of curved molars, upper wisdom teeth, obliterated canals, and teeth covered by crowns, Periodontitis, Cysts, Osteolysis, Pulpitis (after pulp devitalizing) (8). According to the manufacturer's instruction before using Cupral®, a root canal should be subjected to small widening (ISO-30) for two-thirds of its length maximally but not closer than 3 mm up to apical foramen. Copper-calcium hydroxide, by any means, should not go out beyond the tooth foramen (8). It is interesting to know what will happen if we completely prepare the root canal before electrophoresis with Cupral® paste.

It should be noted that a small amount of copper can cause toxicity in adjacent tissue (9). Based on the literature, scarce information exists on the penetration amounts of copper ions through the apex in human teeth. So, this *ex-vivo* study was designed to determine the extrusion of copper ions due to use of a Depotphorese® device through the apex in human single canal teeth.

MATERIALS AND METHODS

Tooth preparation

Current investigation performed on human extracted teeth that teeth have removed due to periodontal or orthodontic problems. The Ethics Committee approved this study protocol under (IR.TUMS.DENTISTRY.REC.1398.118). From all samples, radiographic images with buccolingual and mesiodistal angles were prepared using digital radiography (RVG 5100, Carestream Dental LLC, and Atlanta, GA, USA). The teeth were evaluated in the terms of number and shape of canal, internal and external analysis, root development, crack, and curvature of canal using digital 2d radiographic images (RVG 5100, Care Stream Dental LLC, and Atlanta, GA, USA). A dental operating microscope (Carl Zeiss OPMI pico, Oberkochen, Germany) was used to investigate the presence of cracks on the root surface with a magnification of 4x.

Inclusion and exclusion criteria

Inclusion criteria were single root, and single canal teeth mature apex. Exclusion criteria were teeth with root crack, internal resorption, external resorption, caries, and more than one canal. The teeth were stored in sodium hypochlorite solution (NaOCl, 5.25%) for 30 minutes following store in normal saline solution. Access cavities were prepared in all specimens. Samples were randomly divided into group 1: with root canal preparation (WP), and group 2: control (without root canal preparation) (n=15). In group 1, working length was measured through the visual method. A k-file # 15 was inserted into the canal until the tip became visible on the apical foramen. Then the working length was applied with 1 mm shorter length. Using the Navigator EVO system (Medin, Nove Mesto na Morave, Czech Republic), root canal preparation was performed up to #30/.06 rotary file accord-

ing to the manufacturer's instructions. During canal preparation, the canals were irrigated with NaOCl (2.5%, 2 ml) after each instrument change. Instrumentation was done within the limits of the root canal without achieving apical patency.

After preparation, root canal irrigated with 1 ml of 17% EDTA solution (Ethylene-Diamine-Tetra-Acetate) (Morvabon, Iran, Tehran) for one minute and then 2 ml of 2.5% sodium hypochlorite was added (Morvabon, Iran, Tehran) to remove the smear layer. Subsequently, washed with 10 cc of normal saline to remove the effects of disinfectants from the canal.

Copper ion penetration

The roots were dried with sterile gauze, and two layers of nail polish were applied over the entire external surface except for 2 mm apical part of the root. Each sample was individually fixed by alginate in a 5 ml sterile Eppendorf tube (Pishgam, Tehran, Iran), contacting the root end with 2 ml distilled water. Then cupral solution was then placed inside the canals. According to the manufacturer instructions, a negative electrode (cathode) inserted into the root-canal space while positive electrode (anode) was located inside the Eppendorf tube in contact with distilled water (Fig. 1) Copper ions derived from the copper paste were mobilized via a low current electric field (1.5 mA/min for 10 min) by Depotphorese® (Gerät Original II, Humanchemie, Germany) The amount of copper around the root in the solution was measured by the spectrophotometric method and supernatant. [Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength (10)]. The extract was prepared in 0.15 mol/L and centrifuged to get the supernatant. Optical copper-supernatant absorption was read at 540 nm, and copper concentration was measured (11).

Data analysis

Data analysis was conducted with SPSS software (ver.20, SPSS Inc., USA). Descriptive statistics, including means, standard deviations, and frequency distributions, were calculated for each subgroup. T-tests were used to compare the copper ion penetration. $P < 0.05$ were considered as statistically significant.

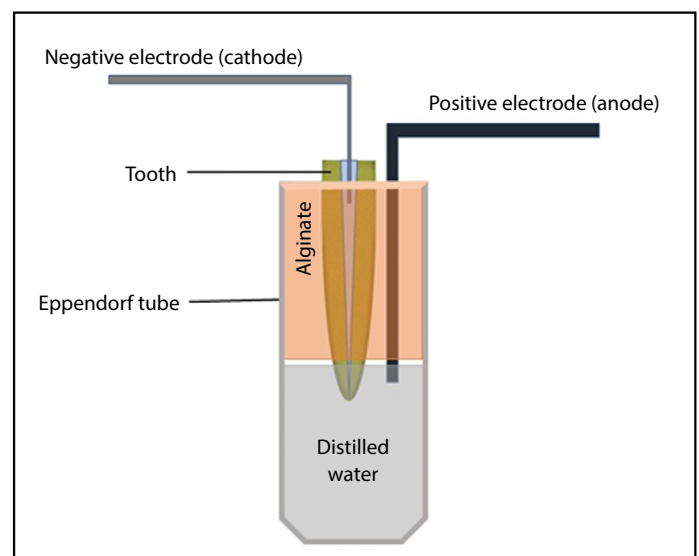


Figure 1. Schematic image of cathode and anode positioning

TABLE 1. Copper ion penetrated in distilled water through the apex of the teeth following the use of depotphoresis

| Group | Mean±SD* | P value |
|-------------------|-------------|---------|
| Spectrophotometry | | |
| Control | 1.58±0.82 | <0.001 |
| WP | 4.14±2.14 | |
| Supernatant | | |
| Control | 28.55±15.07 | <0.017 |
| WP | 50.14±29.20 | |

WP: with root canal preparation, *: (µg/mL)

RESULTS

As a result of the analysis, copper ions were extruded from the end of the apex into two groups. Even though, based on the results of the t-test, copper ion penetration was significantly lower in WOP compared to WP group ($P < 0.05$, Table 1).

DISCUSSION

Endodontic treatment must be done within the confines of the root canal system, to avoid instruments and filling materials from the periradicular tissues (12). An apical constriction is a part of the root canal and located at 0.5 to 1.5 mm from the major apical foramen (13). The preservation of the apical constriction supports endodontic instruments, chemicals, and filling materials within the root canal (13). According to our results, copper ion penetration was significantly lower in the control compared to WP group.

Copper alloys acting on abiotic surfaces showed that besides the rapid death of antibiotic-resistant strains, it also destroyed plasmid and genomic DNA, which implied preventing the spread of infections and gene transfer (14). The starting point for the clinical use of copper, specifically for disinfecting the root canal system, has been reported in the *ex-vivo* study where the canals treated with copper sulfate pentahydrate showed a reduction of 6 logarithms in the count of colony-forming units on the fourth day (15). The results of a biosafety comparing the toxicity of two different copper compounds (CuO nanoparticles, and soluble CuSO_4) revealed a dose-dependent manner. In addition, both compounds caused DNA damage, which was more pronounced in cells treated with CuSO_4 (16).

Studies have shown that the acute lethal dose for adults is based on data from accident and suicide cases ranging from 4 to 400 mg of Copper (II) per kg body weight (17-19). In the present study, the amount of copper released from the apical foramen was below the toxic limit. Electrophoresis, including iontophoresis, can directly transport ions in teeth by utilizing the direct electric field with a low amperage of direct electrical current (20). Electrophoresis causes polar therapeutic molecules to reach the surface of the skin about 10 to 2000 times more than the normal use of a drug (21) In sterilization of the root canal in Rumiantsev's study, the use of calcium hydroxide with electrophoresis has been suggested and this method is recommended to improve healing of apical periodontitis (22).

One of the advantages of electrophoresis and cupral is the cost-effectiveness. In our study, the use of electrophoresis in

both groups resulted in the release of copper ions beyond the apical foramen. According to the current study, electrophoresis can increase the penetration of antimicrobial substances into the root canal (11).

In this study, supernatant and spectrophotometry techniques were used to measure the amount of copper extracted from the apical foramen. In chemistry, spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength (10). The extract was prepared in 0.15 mol/l and centrifuged to get the supernatant. Optical copper-supernatant absorption was read at 540 nm, and copper concentration was measured) (23).

The size of the apical foramen and apical constriction (which vary amongst different teeth) is one limitation of the current study. More studies are needed to examine the amount of ions released in teeth with immature apices and apical root resorption. Furthermore, since this study was performed on extracted teeth, the effect of periapical tissues and periodontal ligament on copper has been eliminated. Future studies would better to be on the focus of copper excretion in animals and examining the effect of copper ions on surrounding tissues.

CONCLUSION

The current study reveals that preparation of root canal increases the copper ion output in extracted human teeth. Electrophoresis caused the copper ion to extrude from the apical foramen which was less than the toxic level. This technology can increase ion release and increase the antimicrobial effect. More studies, are necessary to compare the apical extrusion of copper ions *in vivo*.

Disclosures

Clinical relevance: It is recommended that Cupral® be used before canal preparation for disinfection. It may also be effective in calcified or blocked canals.

Conflict of interest: The authors deny any conflicts of interest related to this study.

Ethics Committee Approval: The ethics committee approved this study protocol under IR.TUMS.DENTISTRY.REC.1398.118.

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