PASSIVE ULTRASONIC IRRIGATION IN CALCIUM HYDROXIDE REMOVAL FROM ROOT CANALS: A SEM/EDS ANALYSIS

ABSTRACT

AIM: The aim of this study was to evaluate the efficacy of different lengths of time of passive ultrasonic irrigation (PUI) in removing calcium hydroxide (CH) paste from root canal, using scanning electron microscopy and energy dispersive spectrometry (SEM/EDS). **MATERIAL AND METHODS:** Fifty-four human premolars were used. After coronal access, a size #15 K-file was used to obtain apical patency. Root canal preparation was performed using WaveOne 40.08 instruments. CH paste was placed into the root canal. Specimens were stored in a humid environment, and after seven days, they were divided into five groups (n=12) according to the irrigation protocols: Manual– a size #40 K-file; PUI/1– 1 min; PUI/2– 2 min; PUI/3– 3 min; and Control– without CH paste. An elementary chemical microanalysis (SEM/EDS) was performed to quantify the presence of calcium on the dentinal walls. **RESULTS:** The percentage of calcium was higher in all experimental groups when compared to the control group ($P < 0.05$); but no differences among them were found ($P > 0.05$). **CONCLUSION:** The amount of calcium hydroxide paste on the dentinal walls was not dependent on length of time of ultrasonic activation. SEM/EDS analysis seems to be a reliable method to assess CH paste removal from the root canals.

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KEYWORDS

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INTRODUCTION

Disinfection using root canal dressing has been proposed in order to reduce the number of microorganisms in the root canal system. Calcium hydroxide (CH) paste has been recommended due to its ability to fight against infections in the root canal system. CH paste has presented some desirable physicochemical properties such as antimicrobial activity, high alkalinity, inhibition of tooth resorption, and tissue dissolving ability.[1-4] To achieve its effectiveness, CH paste must be adequately placed and condensed within the root canal system.[5-6]

However, on the second appointment, this medication must be completely removed from the root canal walls. The presence of calcium hydroxide on dentinal walls could compromise the endodontic treatment.[7] Persistence of CH residues may interfere with the sealing ability of endodontic sealers and their bond strength to the root dentin.[8-10]

Several methods and irrigation substances have been proposed to remove CH pastes from root canals.[8-10] Previous studies have shown that the isolated use of sodium hypochlorite (NaOCl) is not sufficient to achieve this goal.[11-13] On the other hand, irrigation was using NaOCl, recapitulation with the master apical file, and final flush using EDTA yielded superior canal cleanliness over the isolated use of NaOCl, EDTA-C, citric acid, and EDTA-T.[13] The use of rotary instruments or passive ultrasonic irrigation (PUI) also enhanced the calcium hydroxide medication removal.[11,14] However, it is not clear whether the increase in the length of time of PUI could improve the removal of CH residues from root canals.

Earlier reports have evaluated the amount of residual CH pastes using scanning electron microscopy (SEM).[15-16] However, none of these studies have used scanning electron microscopy and energy dispersive spectrometry (SEM/EDS) to perform a quantitative chemical analysis of residual calcium (i.e. chemical element) on the dentinal walls, after using techniques or irrigants to remove CH pastes from the root canal.

The aim of this study was to evaluate the NaOCl efficacy at different lengths of time (1, 2, and 3 min) of ultrasonic activation in removing CH residues from the root canal using SEM/EDS. The null hypothesis was that the amount of calcium on dentinal walls would be similar irrespective of the protocol used to remove CH paste.

MATERIAL AND METHODS

ROOT CANAL PREPARATION AND EXPERIMENTAL GROUPS:

This study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul. Fifty-four single-rooted premolars presenting complete root formation, absence of internal or external resorption, and absence of previous endodontic treatment were used.

All crowns were removed with a double-sided diamond disc (Komet, Santo André, SP, Brazil) using a low-speed handpiece (Kavo, Joinville, SC, Brazil). Root canal length was standardized to a length of 15 mm. The roots were clamped in a metal vice (IBT Machining, Piracicaba, SP, Brazil) to enable the endodontic procedures. All the root apexes were sealed with utility wax (Wilson, Polidental Indústria e Comércio Ltda, Cotia, SP, Brazil) in order to avoid the overflow of the irrigant and to simulate a closed irrigation system.

Initially, a size #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was used to obtain the apical patency. The working length (WL) was established at 1 mm from the apical foramen. The chemo-mechanical preparation was performed using WaveOne 40.08 reciprocating files (Dentsply-Maillefer), in a packing motion until reaching full WL. A reciprocating motor (Silver Reciproc; VDW, GmbH, Munich, Germany) with a 6:1 reduction handpiece was used and assembled as recommended by the manufacturer. Each canal was completely filled with 2.5% sodium hypochlorite (NaOCl) (Biodynamics Inc., Ibiporã, PR, Brazil) before root canal preparation. The solution was delivered into the root canal using a disposable syringe (Injex, Ourimbah-SP, Brazil) with a 29-G needle (Navitip; Ultradent, South Jordan, UT, USA). When the instrument found resistance to reach the WL, it was removed from the root canal, cleaned with wet gauzes, and root canal irrigation using 2.5% of NaOCl was performed. A total volume of 6 mL of 2.5% NaOCl was used to irrigate each specimen during canal preparation. After root canal preparation, a final flush with 3 mL of 17% EDTA (Quinta - Essência, Porto Alegre, RS, Brazil), followed by one minute of passive ultrasonic activation (three periods of twenty seconds) was performed. Finally, root canals were irrigated with 2 mL of 2.5% NaOCl.

Root canals were dried using absorbent paper points, and calcium hydroxide powder was mixed with propylene glycol (Científica, Santa Fé do Sul, SP, Brazil) at a ratio of 0.5g / 0.5 ml. This CH paste was placed into root canals using a size #2 lentulo spiral (Dentsply-Maillefer). Periapical radiographs were taken to confirm if the canals were completely filled with CH paste. The coronal access was sealed with a temporary restorative material (Coltosol; Coltene, WholeDent, Switzerland). All specimens were kept in a humid environment, and after seven days, they were randomly divided into five groups (n = 12), according to the protocol used to remove the calcium hydroxide paste: Manual– a size #40 K-file; PUI/1– PUI for 1 min; PUI/2- PUI for 2 min; PUI/3- PUI for 3 min; and Control- without calcium hydroxide paste.

CALCIUM HYDROXIDE REMOVAL PROTOCOLS:

The calcium hydroxide (CH) removal protocols were: 1. Manual: CH was removed using conventional irrigation (syringe and needle) with 3 mL of 2.5% NaOCl and a size #40 hand file. Then, root canals were irrigated with 3 mL of 17% EDTA for 3 minutes, followed by a final flush with 2 mL of 2.5% NaOCl. 2. PUI/1: CH paste was removed using 3 mL of 2.5% NaOCl, and passive ultrasonic irrigation (PUI) was performed for 1 minute (three periods of 20 seconds). 3. PUI/2: After initial irrigation with 3 mL of 2.5% NaOCl, PUI was performed for 2 minutes (three periods of 40 seconds). 4. PUI/3: After initial irrigation with 3 mL of 2.5% NaOCl, PUI was performed for 3 minutes (three periods of 60 seconds).

Between each period of ultrasonic activation, 1 mL of irrigating solution was used. After PUI, root canals were irrigated with 3 mL of 17% EDTA, for 3 minutes, and flushed with 2mL of 2.5% NaOCl.

PUI was performed using NAC Plus device (Adiel Ltda, Ribeirão Preto, SP, Brazil) by inserting a smooth ultrasonic tip (E1 Irrisonic; Helse, Capelli e Fabris, São Paulo, SP, Brazil), with a similar diameter to a size #20 K-File, at the frequency of 30 kHz, intensity of 7.5 W, with an amplitude between 20 and 30 micrometers, positioned 1 mm short of the WL.

The ultrasonic tip was directed to the center of the root canal to avoid contact with the dentinal walls.

SEM/EDS ANALYSIS:

After the application of the different irrigating protocols for CH paste removal, specimens were fractured longitudinally. Each hemisection was mounted on a metal stub and stored in an oven at 36°C for 48 hours. The samples were carbon coated (Bal-tec SCD 050, Tokyo, Japan) and then assessed using SEM (JSM 5800, JEOL, Tokyo, Japan).

All samples were analyzed using energy dispersive spectrometry (Inca software, Oxford, UK, England) at ×85 magnification within a pre-determined area (250 μm^2) at the apical third. The identification of the calcium (i.e. chemical element) was made within this area using the square tool of the NORAN System SIX software V.1.8 (Figure 1). Therefore, it allowed quantifying the percentage of the calcium on the dentinal wall.

In addition, SEM images were taken with 500× magnifications to illustrate the experimental groups. Data were statistically analyzed using SPSS (SPSS Inc., Chicago, IL, USA) software. ANOVA and Tukey's post-hoc test were used to compare the percentage of calcium found after SEM/EDS assessment. The level of significance was set at 5%.

RESULTS

The percentage of calcium observed after SEM/EDS assessment demonstrated higher amount of calcium in all experimental groups, when compared to the Control group ($p<0.05$) (Table 1). However, the amount of calcium within the root canal walls was similar for all proposed protocols ($p<0.05$). Figure 2 illustrates the presence of residual calcium hydroxide for all the experimental groups.

Figure 1. (A) SEM/EDS analysis (85× magnification) within a pre-determined area (250 μm2) at the apical third. (B) Peaks of calcium (i.e. chemical element) within the assessed area using the square tool of the NORAN System SIX software V.1.8.

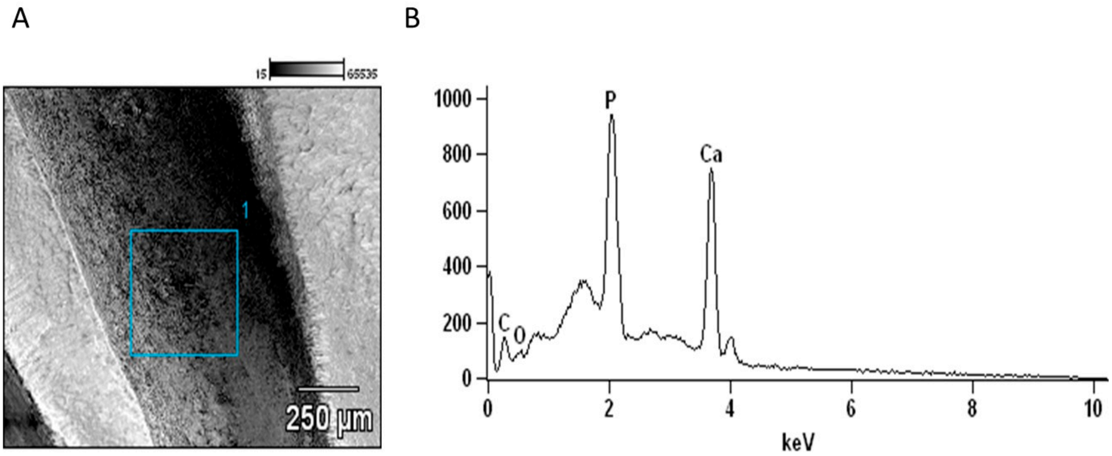


Table 1. Mean and standard deviation (SD) of percentage of calcium obtained after SEM/EDS analysis.

Groups	Manual	PUI/1	PUI/2	PUI/3	Control
Mean	46.55[A]	46.39[A]	42.86[A]	45.19[A]	30.14[B]
SD	6.47	6.18	5.35	8.25	8.11

*Different capital letters are significantly different with a level of significance of 5% (*p*<0.05).

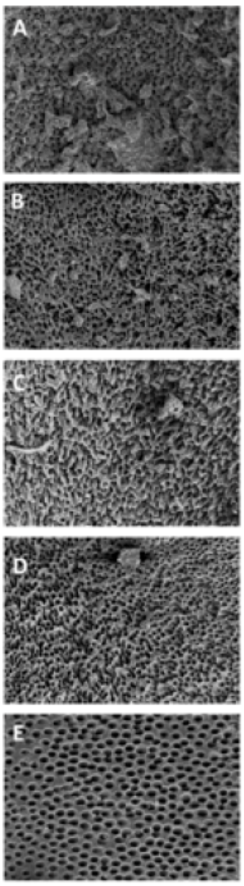
DISCUSSION

An important aspect of the methodology of this study was the use of energy dispersive spectrometry (EDS) to assess CH paste removal from root canals, not yet reported in the Endodontic literature. The JSM5800 microscope consists of an analytical SEM that aims to evaluate organic and inorganic samples. This microscope operates from 0.1 to 30 kV with maximum resolution of 3 nm tension and can be used in conventional observations of secondary electron images, in backscattered electron images and in elementary chemical microanalysis.

SEM/EDS analysis allowed to delimit an area in the apical third of the root canal and to quantify the presence of calcium on the composition of the dentin surface. It should be highlighted that in all the experimental groups both calcium from hydroxyapatite and from CH paste were detected by SEM/EDS. Thus, the presence of a control group, with the absence of CH paste, allowed inferring that the values achieved by SEM/EDS can be attributed to the calcium present on the hydroxyapatite. All experimental groups presented higher amount of calcium when compared to the control group.

On the other hand, no differences between PUI and the Manual group protocols were found. Contrary to the present results, Kenee et al.[11] (2006) have compared some techniques used to remove calcium hydroxide from root canals. PUI and conventional irrigation using NaOCl associated with rotary files removed significantly more residues than hand file and hand file plus EDTA techniques.

Figure 2. Representative SEM images (500× magnification) from experimental groups: A - Manual, B - PUI/1, C - PUI/2, D - PUI/3, and E - Control.



None of the techniques used in this study were capable of removing completely CH from root canals. This is in agreement with previous reports, which showed the presence of calcium hydroxide debris on root canal walls. [8,11-12,14] A considerable proportion of apical third remained filled with calcium hydroxide in C-shaped root canals after instrumentation and conventional needle irrigation.[17] Although the association of rotary instrumentation and sonic or ultrasonic irrigation reduced the amount of residual calcium hydroxide in C-shaped canals, the large amount of calcium hydroxide in the critical apical area remains a concern.[17]

Several studies have shown the presence of calcium hydroxide on dentin walls. [9,17-23] Maximal removal of CH paste from root canals assumes particular importance once it can interacts with zinc oxide-eugenol sealers producing calcium eugenolate.[9] These residues could influence the adhesion of endodontic sealers to the root canal walls, compromising the quality of the seal provided by root fillings. [10,24-26] Barbizam et al.[10] (2008) found a negative effect of calcium hydroxide plus saline and 2% CHX dressings on the adhesion of Epiphany™ to the root canal walls. Additionally, CH paste can compromise the adhesiveness of fiber posts to root dentin.[27] Lee et al.[27] (2014) stated that calcium hydroxide dressing adversely affected the bond strengths of one etch-and-rinse and two self-adhesives cements to root dentin.

The removal of CH residues has been investigated using different irrigants such as NaOCl, citric acid and EDTA in order to dissolve inorganic particles of the smear layer and root canal medications.[8,28] Rodig et al.[12] (2010) stated that the use of chelating agents (10% citric acid and 20% EDTA) promoted the best removal of calcium hydroxide from root canals. The authors explain these results because NaOCl has limited ability to dissolve inorganic substances such as calcium hydroxide. Salgado et al.[13] (2009) associated the use of irrigants (17% EDTA, 0.5% NaOCl, Endo-PTC) with the instrumentation with the master apical file and achieved the highest cleanliness of the root canal after CH placement.

The influence of irrigation time on the efficacy of PUI remains unclear. While one study points to an increased removal of the smear layer after 5 min of PUI in comparison to 3 min,[29] another study indicates a similar dentin debris removal from the root canal after 30 and 60 seconds of PUI.[30] It must be highlighted that the method used by other authors[30] was similar to that used in the present study, in which NaOCl was injected into the root canal using a syringe and refreshed only after PUI ultrasonic activation. Probably, for this reason, no differences were found between the lengths of time of PUI used in this study. Finally, the only data available regarding the effect of the duration of ultrasonic activation on canal cleanliness were obtained over debris and smear layer.[29-30]

The major advantage of this experimental model is that residual CH paste can be assessed in a greater area in comparison with conventional SEM. Conventional SEM uses 1000× and 2000× magnifications, and represents only restricted areas of one specimen, which may not be representative of the entire sample. In other studies that used stereomicroscopy and SEM with different magnifications, the calcium hydroxide residues were evaluated using scores.[13,15,18-19,31] Scores have been questioned because when this type of assessment is performed, usually a 2000× magnification is used. Therefore, only a small area of the specimen is evaluated, and it may not be representative from what occurs on the entire sample. Instead, when SEM/EDS are used, a chemical analysis is performed on the entire sample. Finally, scores may present variations inside the same score level. For example, scoring methods to assess the presence of debris indicating less than 50% of tubules visible may contain debris ranging from 1% to 50%. All this variation represents the same score. In SEM/EDS analysis, the evaluation is expressed quantitatively in percentage of the chemical element at 85× magnification.

CONCLUSION

This study was not able to show complete removal of calcium hydroxide residues from the canal walls under the conditions tested, regardless of the protocol used. The amount of calcium hydroxide paste on dentinal walls was not dependent on the time duration of ultrasonic activation. Finally, SEM/EDS analysis seems to be a reliable method to assess the removal of CH pastes from root canals.

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