Comparison of Sonic and Ultrasonic Activation for Removal of Calcium Hydroxide from Root Canals - A Micro-Ct Study

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Abstract
Residual calcium hydroxide in the root canal can affect the quality of the root canal filling. The purpose of the present study was to use microtomography (micro-CT) for evaluating the efficacy of sonic and ultrasonic activation in removing calcium hydroxide.

Thirty-two single-rooted mandibular premolars were prepared using a ProTaper next to X3 and were filled with a calcium hydroxide paste. The teeth were divided into two groups (n = 16) and an initial scan was subsequently performed using micro-CT. Following a seven-day incubation under 100% humidity at 37°C, the calcium hydroxide was cleaned with an irrigation solution that had been activating using either a sonic (EDDY™, VDW) or an ultrasonic instrument (IrriSave, Acteon Satelec). After cleaning, a second with micro-CT scan was performed for determining the volume of residual calcium hydroxide. The data were reconstructed and analyzed using the NRecon and CTAn software.

The ultrasonic group had a lower mean residual volume of calcium hydroxide than the sonic group; however, the difference was not statistically significant (p = 0.225). Sonic and ultrasonic techniques have the same ability to remove calcium hydroxide from the root canal.

Introduction
Calcium hydroxide is used in numerous endodontic treatments as a pulp cap material, root canal medicament, root canal sealer, and medicament in root resorption cases. The lethal effects of calcium hydroxide on bacterial cells are caused by protein denaturation, causing damage to bacterial DNA.1,2 Its mechanism of action is the release of Ca2+ ions, which play a role in the tissue mineralization process, and OH-ions which exert an antimicrobial effect by increasing the pH to form an alkaline environment. In addition, its insoluble nature in water can be exploited for providing long-term medicament.3 Calcium hydroxide is able to eliminate almost all endodontic bacteria and can act as an effective anti-endotoxin agent if applied to root canals for 7 days.4,5

Prior to the obturation of the root canal, the medicament material should be cleaned from the root canal wall, since the presence of calcium hydroxide residue can affect the success of endodontic treatment, causing sealer leakage in the apical region. Moreover, calcium hydroxide residue can dissolve over time and cause a gap between the walls of the root canal and the sealer. Sokhi et al. showed that residual calcium hydroxide containing 2% chlorhexidine resulted in micro-leakage of resin-based sealer in one-third of the apex.6,7

The irrigation solutions used for removing residual calcium hydroxide are EDTA, NaOCl and a combination of both. Salgado et al. recommended the cleansing of calcium hydroxide residue using a combination of 17% EDTA followed by irrigation with NaOCl, will resulted in a cleaner root canal wall as compared with the use NaOCl alone.7 A commonly used method for removing calcium hydroxide medicaments is the use of a master apical file combined with irrigation fluid using conventional irrigation needles.8,9 To date, there is no technique or tool able to thoroughly clean
the calcium hydroxide from the root canal. Several studies have shown that a negative-pressure irrigation system (Endovac) and sonic agitation systems (Endoactivator™), and passive ultrasonic irrigation (PUI) are more efficient at cleaning calcium hydroxide from the root canal than irrigation using conventional irrigation needles.10,11.

Recently, EDDY™ (VDW, Munich, Germany), a novel sonic activation device was developed, which consists of a flexible polyamide irrigation tip that is activated using a 5000–6000 Hz air scaler.12 It can create three-dimensional movement that triggers the effects of cavitation and acoustic streaming, greatly improving cleaning efficiency.13 In the present study, the effectiveness of sonic and ultrasonic irrigation techniques in removing calcium hydroxide from root canal walls was analyzed using micro-CT.

Materials and methods Sample selection and pre-mechanical preparation

Following approval by the Dental Research Ethics Committee (KEPKG) of the Faculty of Dentistry, Universitas Indonesia, 32 specimens of mandibular premolar teeth extracted for orthodontic reasons were randomly selected. The sample had single root canal that has an average length of 20 ± 2 mm, intact apices, and no root caries or defects (resorption and calcification).

All specimens were cleaned with an air scaler, and immersed in of 0.9% NaCl solution until use. The teeth were subsequently examined by radiography on the mesiodistal aspect to observe the anatomy of the root canal. The teeth were numbered and divided into two groups, with 16 samples in each group, and subsequently placed in a study container filled with and saline solution for soaking, which were numbered according to the sample number on each tooth.

The root canal patency was verified by placing a #15 K-file through the apical foramen, and the working length was established by substracting 0.5 mm from this measurement. The root canal preparation was performed with a ProTaper next to X3 at 300 rpm, and simultaneously, all root canals were irrigated with 2 mL 2.5% NaOCl using a 30-G side-vented irrigation needle inserted 1 mm short of the working length into the root canal following each preparation cycle. Five millimeters of 17% EDTA solution was used following root canal preparation to remove the smear layer. The root canal was dried using a sterile paper point, followed by the injection of calcium hydroxide paste until the material extruded through the apex. Confirmatory radiography was performed in the mesiodistal and buccolingual directions to confirm complete filling of the root canal. The access cavities were temporarily sealed with a cotton pellet and temporary fillings.

The teeth were stored in the coronal position in a container filled with moistened cotton soaked in saline. The samples were subsequently stored in an incubator at temperature of 37° C and humidity of 100% for 7 days, following which the initial micro-CT scans were performed.

Irrigation technique

Following removal of the temporary filling material, specimens were divided into two groups of 16 teeth. A #15 K-file and with 3 mL 2.5% NaOCl were used to remove the bulk of the calcium hydroxide. All root canals were irrigated following a standardized protocol that included 6 mL 17% EDTA followed by 6 mL 2.5% NaOCl using a 30-G needle at 1 mm from the working length. The volume and time were standardized for each group.

Group 1: Sonic irrigation

The removal of calcium hydroxide was performed by sonic irrigation using a 6000 Hz air scaler coupled with EDDY™. The tip was inserted into the canal and activated at 2 mm from the working length. Sonic activation was delivered twice for 60 seconds during 17% EDTA and once during 2.5% NaOCl irrigation.

Group 2: Passive ultrasonic irrigation technique (PUI)

PUI was performed using a Satelec P5 Neutron ultrasonic unit and IrriSave file #20 for 60 seconds. The ultrasonic unit was maintained at a power setting of 6. The IrriSave tip was inserted 2 mm from the working length, and no agitation was performed during activation. PUI activation was delivered twice for 60 second during 17% EDTA irrigation, and once during 2.5% NaOCl irrigation.
Following both types of irrigation, the teeth were dried with a sterile paper point and covered with sterile cotton. All procedures were performed by the same operator.

**Scanning with micro-CT and data analysis**

Scanning was performed using a SkyScan 1173 (Bruker, Belgium). All samples were scanned in high-resolution (1 pixel = 29.8 μm) with a rotation step 0.2° for 2 hours. Two micro-CT scans were performed; one following insertion of the calcium hydroxide and one following removal of the calcium hydroxide. The scanning results were two-dimensional images, which were subsequently reconstructed into 3D images with the help of the NRecon and DataViewer software (Bruker, Belgium). Images were analyzed with the CTAnalyzer software (Bruker, Belgium) to determine the volume of calcium hydroxide present prior to and following cleaning. The volume of calcium hydroxide was calculated from the binarized area inside the region of interest in mm3.

**Statistical analysis**

A t-test was used to compare sonic and ultrasonic groups using the SPSS v21 software. The Mann–Whitney U test was performed to compare the sonic and ultrasonic groups, since the data failed to pass the normality test (significance level, p <0.05)

**Results**

The results of the present study show that a residual volume of calcium hydroxide remained in the root canal following activation with both sonic and ultrasonic irrigation (Figure 1 and 2). A normality test was performed on the data rom both groups, obtaining a p value < 0.05 and concluding that the data was non-parametric. A Mann – Whitney test was subsequently conducted to determine significance. The median volume of calcium hydroxide residue present following activation with sonic and ultrasonic irrigation was 0.006 and 0, respectively (Table 1). The Mann – Whitney significant test obtain a value of p = 0.225, showing that there was no statistically significant difference in volume of residual calcium hydroxide between the two groups.

<table>
<thead>
<tr>
<th>Irrigation group</th>
<th>n</th>
<th>Median</th>
<th>Min – Max Value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sonic</td>
<td>16</td>
<td>0.006</td>
<td>0 - 0.057</td>
<td>0.225</td>
</tr>
<tr>
<td>Ultrasonic</td>
<td>16</td>
<td>0</td>
<td>0 - 0.030</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Average residual volume of calcium hydroxide residues (mm3) between two groups.

![Image](a) ![Image](b) ![Image](c)

**Figure 1.** Reconstructed micro-CT image of premolar teeth in the sonic group, (a) Calcium hydroxide application following instrumentation; (b) following the removal of calcium hydroxide; (c) cross-section of the lower 1/3 of the apex, the ed region shows region of interest (ROI) calcium hydroxide residue.

![Image](a) ![Image](b) ![Image](c)

**Figure 2.** Reconstruction micro-CT images of premolar teeth in the ultrasonic group, (a) Calcium hydroxide application following instrumentation; (b) following the removal of calcium hydroxide; (c) cross section of the lower 1/3 of the apex with no residual calcium hydroxide following removal.

The sonic group had a greater median value than the ultrasonic group, indicating that the residual volume of calcium hydroxide in the sonic group was larger. It can be deduced that,
although the ultrasonic technique cleared the calcium hydroxide substantially better than the sonic technique, there was no significant difference between the two groups.

Discussion

The present study aimed to determine the difference in effectiveness between sonic and ultrasonic techniques in removing calcium hydroxide residue from the root canal, as analyzed by micro-CT; an imaging method that is non-damaging and provides accurate volume analysis as compared with measuring surface area in two-dimensional space.\textsuperscript{14}

The teeth used in the present study were mandibular premolars, with radiographically confirmed single root canals, with the aim of obtaining a uniform sample and easy preparation. In addition, premolars are easy to obtain, since they are often indicated for extraction in orthodontic treatment. The criteria for the teeth were an apical diameter equal to the K-file ISO #15 and a determined working length of 0.5 mm from the apical foramen, equalizing the cross-sectional area of the canal across the teeth.

In the present study, the root canal was injected with ready-to-use calcium hydroxide, due to its practical and easy application; this form is different from purified calcium hydroxide, which can turn into calcium carbonate when in contact with carbon dioxide in the air, for too-long a period, becoming ineffective. The ready-to-use paste has a composition of calcium hydroxide, barium sulfate, and propylene glycol, and has a high radiopacity due to the barium sulfate; thus, it is easy to perform radiographic evaluation.\textsuperscript{15,16} The calcium hydroxide was incubated for 7 days at 37° C under humid conditions resembling the oral cavity. In clinical applications, the minimal use of calcium hydroxide is for 7 days. According to Kamran et al., the antibacterial effects of calcium hydroxide effective in the hydrolisis of Gram-negative bacterial LPS t in the root canal for at least 7 days.\textsuperscript{1,5}

The calcium hydroxide paste is an inorganic material used as a root canal medicament in endodontic treatment, however, a disadvantage of such a medicament is the accumulation of particles in the root canal. If there is a failure to remove the calcium hydroxide particles from the root canal, it will cause difficulty for the sealer to penetrate into the dentine tubule, and may cause voids between the root canal wall and the sealer, resulting in failure of the root canal treatment.\textsuperscript{17} Porkaew et al. stated that of calcium hydroxide residue can combine with eugenol containing sealers, causing the permeability of the sealer to decrease. In addition, if particles of calcium hydroxide residue enter the dentin tubule, dentine permeability will also be decreased.\textsuperscript{18}

The irrigation materials used in the present study were 2.5% NaOCl and 17% EDTA. NaOCl has an antimicrobial action and can dissolve organic tissue. According to Christensen et al., using of NaOCl at concentrations of 5.25% and 2.625% showed no significant difference in its ability as an antimicrobials, while at concentration of 0.5% and 1% was sufficient as a disinfectant, but not sufficiently effective at dissolving organic tissue.\textsuperscript{19} EDTA at a concentration of 17% was used as a chelator, a material capable of attracting metal ions such as chemically binding calcium to enhance cleaning during root canal preparation. Combining the use of NaOCl and EDTA as an irrigation solution have a synergistic effect, and has been recommended for removing calcium hydroxide.\textsuperscript{20,21}

The activation of irrigation solution is known to increase debris removal from the root canal. In the present study, we compared sonic and ultrasonic techniques for removal of calcium hydroxide medicaments. Several studies have shown that the cleansing of calcium hydroxide medicaments using ultrasonic irrigation is able to clean better than any other technique.\textsuperscript{22,23,24} The use of ultrasonic irrigation techniques was recommended several years ago; this instrument has a generated frequency ranging from 25,000 - 30,000 Hz, and the ultrasonic tip used in this present study was of a size comparable to a K-file #20 so as not to cut the dentin in the root canal wall. During activation, the ultrasonic tip oscillates, activating the irrigation solution and causing fluid movement known as acoustic streaming, which forms a cavitation, i.e., the formation of air bubbles when the fluid receives high energy. The presence of such effect removes microorganisms, dentin debris, and organic tissue from the root canal. According to Ahmad et al., the use of files larger than #20 has a smaller acoustic stream effect, since small files easily oscillate in the root canal
transmitting their energy to the irrigation solution.\textsuperscript{13,25}

In contrast, sonic techniques are operated at lower frequencies (1-6000 Hz). The present study used the latest sonic activation system with the EDDY™ tip (VDW); its flexible polyamide tip, with a size of 25.04 can be coupled to an air scaler that operates at 5000-6000 Hz. It has advantages such as noncutting, sterility, low risk of fatigue fracture on the tip, and does not cause a ledge in the root canal.\textsuperscript{24}

The results of the present study show that in certain samples from both groups, some calcium hydroxide still remained, which is similar in accordance with previous studies using different instruments and methods.\textsuperscript{8,10,26} In the present study, residual calcium hydroxide in both groups was present in the lower 1/3 of the apex, since there is a decrease in cleaning efficiency associated with the shrinking diameter of the root canal (Figure 1 and 2). Lee et al., suggested that root canal diameter has an impact on the effectiveness of debris removal, the volume of irrigation solution, and fluid movement in the lower 1/3 apex.\textsuperscript{27}

In research conducted by Wiseman et al., the use of the passive ultrasonic irrigation (PUI) technique was shown to remove calcium hydroxide significantly better as compared with sonic (EndoActivator) techniques,\textsuperscript{23} however, there has been no study comparing calcium hydroxide clearance between sonic activation systems (EDDY™) and ultrasound. In the present study, it was found that of the 16 samples in the ultrasonic group, 11 had a value 0 (no residue of calcium hydroxide in the root canal), whereas in the sonic group, only 8 samples had a value 0; however, this difference was not statistically significant. This is likely due to the sonic technique causing a streaming effect, although sonic instruments are operated at frequencies below those of ultrasonic instruments. This streaming effect occurs due to an oscillating movement of the tip at high amplitudes, while in ultrasonic files vibrate at a high frequency with nodes and antinodes throughout the vibrating tip.\textsuperscript{26}

The sonic techniques used in the present study, visually activated irrigation fluids and produced greater water splashes as compared with PUI-generated aerosols, therefore, the administration of an irrigation solution should be intermittent using a syringe to avoid drying of the root canal due to strong activation effects. With the root canal being continuously filled with irrigation solution, the hydrodynamic movement in the root canal continues to be generated upon activation.

There exist several methods to evaluate the cleanliness of root canal walls with respect to calcium hydroxide medicament residues, such as stereo microscopy, using digital photography, scanning electron microscope (SEM), micro computed tomography (micro-CT). In a previous study, stereo microscopy was used to examine the cleanliness of the root canal wall, but this method is considered less accurate, since debris can enter during the splitting of teeth, affecting the results.\textsuperscript{14}

Micro-CT is able to measure the volume and percentage of residual calcium hydroxide remaining on the root canal wall. This system provides a 3D image from which the location of the residual calcium hydroxide can be determined, and the volume of residual calcium hydroxide can be calculated in mm3 with the help of the CTAnalyzer (CTAn) software.\textsuperscript{23} In addition, micro-CT facilitates the evaluation of the cleanliness of the root canal without damaging the tooth, providing better analysis results than those obtained from the usual scoring method used for SEM examination. The disadvantage of SEM scoring method is that does not permit a highly sensitive quantitative comparison of the experimental groups, and there is variation between observers when evaluating images.

Conclusions

Combined irrigation of 17% EDTA and 2.5% NaOCl activated using sonic and ultrasonic techniques have the same ability to remove the calcium hydroxide medicaments residue from the root canal.

Declaration of Interest

The authors deny any conflicts of interest related to this study. The publication of this manuscript is supported by Universitas Indonesia.
References