



## ORIGINAL ARTICLE

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## Sonic versus ultrasonic activation for removal of calcium hydroxide from curved root canals: an *in vitro* study.

**Abstract:** To compare the remaining calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) coated area in the apical third of curved canals after sonic and ultrasonic activation using Scanning Electron Microscope (SEM). Permanent mandibular molars were collected. Those which radiographically presented a curvature between  $15^\circ$  and  $30^\circ$  in the mesial root were included. A total of 39 mesiolingual canals were instrumented with rotary ProTaper and filled with  $\text{Ca}(\text{OH})_2$  mixed with propylene glycol. They were randomly divided into three groups according to the type of removal. Group I: three inserts with File F2 and 2ml of 5.25% sodium hypochlorite (NaOCl) irrigation between each insertion. Group II: three sonic activations, irrigation with 2ml of 5.25% NaOCl between activations. Group III: three ultrasonic activations irrigating with 2ml of 5.25% NaOCl between activations. Three root canals were left without filling and other three were completely filled as positive and negative control, respectively. Then, they were fractured lengthwise to get two sections. The apical third of the root canal of each section was taken x-rays with SEM and subsequently, in an area of  $100,000 \mu\text{m}^2$ , the remaining  $\text{Ca}(\text{OH})_2$  coated surface from both sections was measured using ImageJ 1.47. They were analyzed with one-way ANOVA using Graph Pad-Prism (5.01). The percentage of the remaining  $\text{Ca}(\text{OH})_2$  coated area in group I was 62.93%, 51.77% in group II, and 58.90% in group III. There were no significant differences between the three groups ( $p > 0.01$ ). There are no significant differences in the percentage of the remaining  $\text{Ca}(\text{OH})_2$  coated area in the apical third of curved root canals when comparing sonic and ultrasonic activation using SEM.

**Keywords:** Endodontics, Calcium hydroxide, removal, Sonic, Ultrasonic.

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### INTRODUCTION.

Calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) is an effective intracanal medicament. Its effects are seen through antimicrobial action, the inhibition of osteoclastic activity and a favorable tissue repair response during endodontic therapy, among other benefits<sup>1,2</sup>.

However, it was demonstrated that  $\text{Ca}(\text{OH})_2$  interacts with eugenol inhibiting the correct zinc oxide eugenol chelate (ZnOE) formation, thus giving ZnOE a fragile consistency and a granular structure and leaving residual eugenol<sup>3</sup>. In addition to this,  $\text{Ca}(\text{OH})_2$ , as an intracanal

medication, can increase the apical leakage of root canals filled with gutta-percha when using ZnOE as a sealer<sup>4</sup>. This is why as much  $\text{Ca}(\text{OH})_2$  as possible must be removed from the root canal<sup>5</sup>.

Among procedural errors causing endodontic failure, the lack of filling is the one with the lowest success rate, especially when there are necrotic pulp and periradicular lesions<sup>6</sup>. Besides, the most common cause of endodontic failure is leakage of the root canal filling<sup>7</sup>.

Different methodologies have been evaluated in vitro for removing  $\text{Ca}(\text{OH})_2$ . Some of them depend on the ve-

hicle used<sup>8-10</sup>, others are related to the irrigant<sup>11-16</sup>, some are based on the technique, comparing removal with rotary instruments and ultrasonic activation<sup>1</sup>, and others comparing sonic activation<sup>17,18</sup> as well. But, no single technique can completely eliminate  $\text{Ca}(\text{OH})_2$ . Therefore, it is relevant to evaluate the remaining amount which is actually remaining in the canal with a microscopic vision, especially if it is curved root canals since it is cited as a factor influencing the elimination of  $\text{Ca}(\text{OH})_2$ <sup>1</sup>.

The objective of this in vitro study is to compare the remaining area with calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) in the apical third of curved root canals after sonic and ultrasonic activation using Scanning Electron Microscope (SEM). The null hypothesis is that the ultrasonic activation is not better than sonic activation.

## MATERIALS AND METHODS.

An *in vitro* experimental study was carried out. First and second permanent molars extracted from patients in the Dental Unit of the "Jorge Sabbath Gozalo" Family Health Center (Centro de Salud Familiar, CESFAM) and France External Clinic were collected prior approval from the Scientific Ethics Committee of the Health Service in Valdivia (ORD: No. 079). They were stored in a solution of 5.25% sodium hypochlorite ( $\text{NaOCl}$ ) (Chemistry Hertz, Santiago, Chile) at room temperature for up to two days. Periodontal tissues were removed with 7/8 Gracey curette (Dentsply Maillefer, Ballaigues, Switzerland), then, they were washed with distilled water and immersed in a solution of 0.9% sodium chloride ( $\text{NaCl}$ ) (B. Braun Medical S.A., Santiago, Chile) and refrigerated at 4°C until use for a maximum time of one month.

Each tooth had X-rays taken from mesiodistal and buccolingual direction to analyze the anatomy of the root canals. Teeth, the radiographic package and cardboard were numerically labeled with a marker to identify each tooth with its respective x-ray. Then, they were visualized by an operator on a negatoscope with 4x magnification in a room with dim light.

The included teeth radiographically presented a curva-

ture between 15° - 30°, with a fully formed apex in the mesial root. The excluded teeth radiographically presented: tooth decay, fractures or cracks, signs of internal or external resorption, calcifications in the duct and/or pulp chamber in this root or had been previously treated.

A sample size in 11 teeth per group was estimated using Epidat 4.0 (Servizo de Epidemioloxía de la Dirección Xeral de Innovación e Xestión da Saúde Pública de la Consellería de Sanidade (Xunta de Galicia)) with a confidence level of 99.9%, statistical power of 99% and effect level of 10% of the area with  $\text{Ca}(\text{OH})_2$ . Other six teeth were positive and negative control: three without  $\text{Ca}(\text{OH})_2$  and three with  $\text{Ca}(\text{OH})_2$ , respectively. All of the teeth were discarded at the end of the study.

The treatment of the samples was based on the protocol of Keene *et al.*<sup>1</sup> which is described below:

Longitudinal grooves were made in the mesial and distal face of 39 mesial roots of mandibular molars, and a transversal furrow of 0.5 mm depth measured with WHO Probe (Dentsply Maillefer, Ballaigues, Switzerland) was made in the boundary between the middle and apical third of the root with #10 diamond fissure bur (SS White, Gloucester, England) mounted on a turbine (NSK Panamax, Tokyo, Japan).

Arabic numerals were sequentially labeled with diamond fissure bur mounted on a turbine (NSK Panamax, Tokyo, Japan).

Endodontic access was carried out and the working length (WL) in the mesiolingual canal of 39 mandibular molars was directly estimated at 1mm from the apical foramen with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland). Then, the apical foramen was sealed with yellow wax (Decocera Ltda., Santiago, Chile). It was set up with rotary Pro-Taper until F2 file (Dentsply, Maillefer, Ballaigues, Switzerland), mounted on Endo-mate DT motor (NSK, Tokyo, Japan), by using the following irrigation protocol: 1ml of 5.25%  $\text{NaOCl}$  between each file and final irrigation with 1ml of ethylenediaminetetraacetic acid (EDTA), liquid solution at 10% (Chemistry Hertz, Santiago, Chile), for one minute and then 1ml

of distilled water. All the irrigants were delivered with a monoject syringe for endodontic irrigation (Kendall, Mansfield MA, USA). It was passively introduced to WL and dried with #30 paper cones (GAPADENT., Korea) after each irrigant.

The mesiolingual canal of 33 mandibular molars was filled with  $\text{Ca}(\text{OH})_2$  podwer (Chemistry Hertz, Santiago, Chile) mixed with Propylene Glycol (Chemistry Hertz, Santiago, Chile) to form a paste which was placed using a #30 lentulo (Dentsply, Maillefer, Ballaigues, Switzerland) mounted in contra angle for hand piece (NSK, Tokyo, Japan).

An x-ray was taken in distolingual and mesiobuccal direction to confirm the total of filling of the canal labeled in the radiographic cardboard with the same number previously labeled in the molar. In case of partial filling, it was filled again until achieving complete filling and the access was sealed with endodontic yellow wax. Subsequently, the molars were kept in 50ml Falcon tubes (Miniplast, Tlalnepantla, Mexico) with 0.9% NaCl at 37°C for seven days.

A simple randomization was conducted through Epi-Dat 4.0 to form three groups, according to the way of removing  $\text{Ca}(\text{OH})_2$ .

Prior to starting the protocols for removing  $\text{Ca}(\text{OH})_2$ , the yellow wax was taken away from the crown, the Protaper F2 file was inserted to WL and was irrigated with 2ml of 5.25% NaOCl for one minute with a monoject syringe until WL or until finding resistance in all teeth. After that, it was preceded as follows:

Group I [rotary Protaper] (n=11): three insertions with F2 file to WL, it was irrigated with 2ml of 5.25% NaOCl for one minute with a monoject syringe until WL or until finding resistance between each activation.

Group II [Sonic Activation] (n=11): three activations for 20 seconds each to WL-1mm or until resistance was felt with a blue 30/06 insert mounted on sonic device (EndoActivator Dentsply York, Pennsylvania, USA) at 10,000Hz per minute, and irrigation with 2ml of 5.25% NaOCl between each activation.

Group III [Ultrasonic Activation] (n=11): three activations for 20 seconds each with E-11 tip mounted on ultrasonic instrumentation (NSK several 350, Tokyo, Japan) at 16,000 Hz per minute up to WL-1mm or until resistance was felt and irrigation was done with 2ml of 5.25% NaOCl between each activation.

Finally, 6 molars were set up and then were randomly assigned into two groups depending on whether they were filled with  $\text{Ca}(\text{OH})_2$  or not and labeled respectively as NC [Negative Control] (n=3) and PC [Positive Control] (n=3).

The 39 roots were longitudinally sectioned in the previously carved cracks with shear. They were wrapped in adhesive plastic sheets (ALUPLAST, Valparaiso, Chile) and kept in a closed Styrofoam box for a maximum time of one week.

Each root had two sections: one lingual (50% of the total area) and one vestibular (50% of the total area). The apical third of the mesiolingual canal of both sections was evaluated with scanning electron microscope (Leo Electron Microscopy 420, Carl Zeiss, Tokyo, Japan) from the Electron Microscopy Department (Research and Development Management, Universidad Austral de Chile, Isla Teja Campus, Valdivia-Chile).

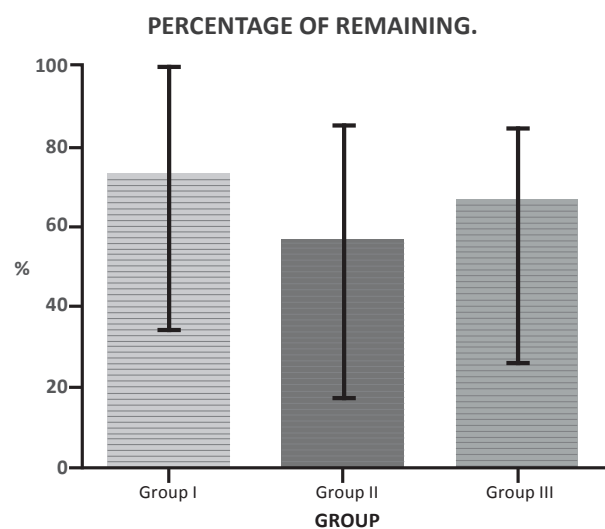
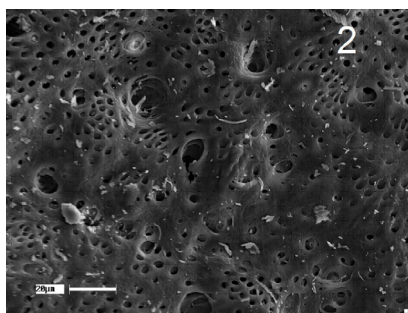
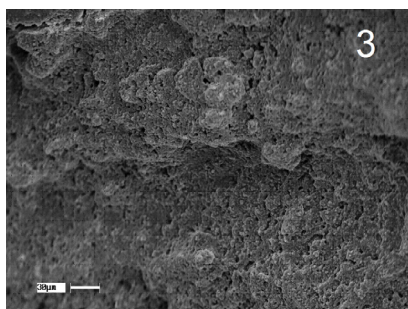


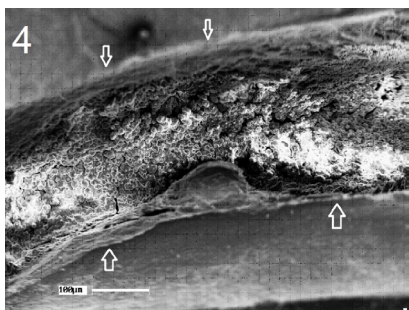
Figure 1. Percentage of remaining  $\text{Ca}(\text{OH})_2$  and its respective standard deviation.



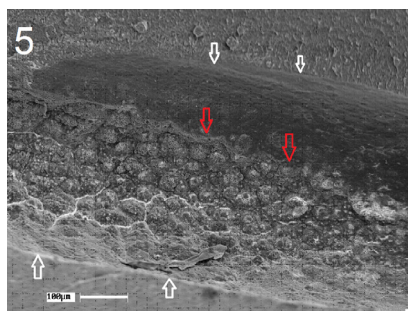
**Figure 2.** Positive control, canal without  $\text{Ca(OH)}_2$  filling.



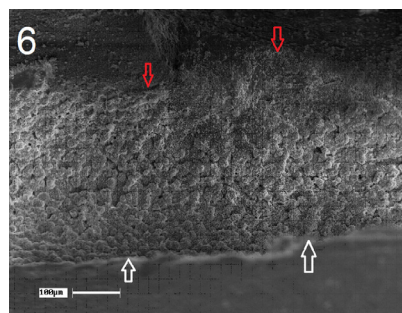
**Figure 3.** Negative control, root canal filled with  $\text{Ca(OH)}_2$ .



**Figure 4.** Group I, removal of  $\text{Ca(OH)}_2$  with rotary Protaper. The arrows indicate the boundary of the root canal. The walls of the root canal are seen to be completely covered with  $\text{Ca(OH)}_2$ .



**Figure 5.** Group II, removal of  $\text{Ca(OH)}_2$  with sonic activation. The white arrows indicate the boundary of the root canal. The red arrows indicate the beginning of remaining areas with  $\text{Ca(OH)}_2$ .



**Figure 6.** Group III, removal of  $\text{Ca(OH)}_2$  with ultrasonic activation. The white arrows indicate the boundary of the root canal. The red arrows indicate the beginning of remaining areas with  $\text{Ca(OH)}_2$ .

Each of the samples was fixed in a design of aluminum using adhesive electrical conductor. Subsequently, samples were coated with a submitted to a 200Å degree layer of gold-palladium. Electron micrographs were taken of the apical third of the mesiolingual canal.

A new labeling was assigned to the images obtained through SEM so that the operator responsible for evaluating them knew the type of removal used. For each section of the root canal, an area of 100,000µm<sup>2</sup> was observed, which gave a total area of 200,000µm<sup>2</sup> per canal. The remaining surface with  $\text{Ca(OH)}_2$  from both sections was measured with using the ImageJ 1.47 program (National Institute of Health (NIH), Maryland, USA) and data were tabulated in a spreadsheet. The sum of the area covered with  $\text{Ca(OH)}_2$  from both sections was calculated and expressed in percentage for each tooth.

Data were analyzed with one way ANOVA test, with confidence level of 99.9% ( $p < 0.01$ ) with GraphPad-Prism 5.01 (GraphPad Software, Inc., California, USA).

## RESULTS.

Figure 1 shows the percentage of the remaining area with  $\text{Ca(OH)}_2$  per group: the percentage of remaining area with  $\text{Ca(OH)}_2$  in group I was 62.93%, 51.77% in group II and 58.90% in group III. The percentage of remaining  $\text{Ca(OH)}_2$  is less in group II, but the difference is not significant ( $p > 0.01$ ). The representative images per group and the ones for the positive and negative control groups can be observed in Figure 2, 3, 4, 5 and 6.

## DISCUSSION.

When comparing the remaining  $\text{Ca}(\text{OH})_2$  between groups, there is less in group II. In spite of the fact that this difference is not significant, the dispersion of data is biased toward lower values than the group with ultrasonic activation.

This was probably due to the fact that the tip of the EndoActivator is more flexible. Therefore, in the case of curved canals, it is easier to enter to a greater length than with the tips of the ultrasound, despite the fact that it vibrates at a lower frequency.

Although the handling of samples followed a similar methodology to previous studies, our results show that there is no significant difference between the use of ultrasonic or sonic activation for removing  $\text{Ca}(\text{OH})_2$ . This differs from the study by Khaleel *et al.*<sup>18</sup> where a significant difference in favor of the use of ultrasonic activation was found. It should be noted that teeth were fractured before withdrawal of  $\text{Ca}(\text{OH})_2$  which, according to Keene *et al.*<sup>1</sup>, decreases measurement accuracy and does not ensure that the canal is free of debris before carrying out interventions. Besides, only single-rooted teeth without curvature were evaluated, so the ultrasonic tip had greater ease to reach greater length in comparison to teeth with curved canals. Other consideration to take into account is that the vehicle used in their study was distilled water and the measuring instrument was the dental surgical microscope (DSM).

Maalouf *et al.*<sup>19</sup> agree with our results since they found no significant differences between the techniques evaluated, but indicate ultrasonic activation is more effective for removing  $\text{Ca}(\text{OH})_2$ . This can be explained, as in the previous study, because they evaluated single-rooted teeth with a clean canal without curvature. In a group, a mixture of  $\text{Ca}(\text{OH})_2$  only with water was also used and the measuring instrument was a Sony DSC-T200 camera.

Wiseman *et al.*<sup>17</sup> used mandibular molars as in the present research and the measurement instrument micro-computed tomography (micro-CT). They found a significant difference favoring ultrasonic activation, which disagrees with our results. They did not specify the level of expected effect. However, judging by the sample size, it must have

been less than the stipulated in our study.

Keene *et al.*<sup>1</sup> also assessed  $\text{Ca}(\text{OH})_2$  remaining in the canal of mandibular molars and, among the techniques investigated, compared removal with ultrasonic activation and rotary Protaper without finding significant differences. Their findings are consistent with our results, although their results were obtained using DSM.

Among the inherent limitations in this type of study, there is the fact that there is not a standardized level of effect, *i.e.* the minimum value which determines that the observed differences are significant considering clinical implications and allowing results vary depending on this information.

On the other hand, specifically in our research, we compared activation methods using only NaOCl as irrigant

Based on the results obtained, the withdrawal of calcium hydroxide is greater when using irrigant activation, although this difference is not statistically significant. It is suggested to perform additional studies to generalize more information for the clinical area.

Experimental studies to standardize a level of effect so that the differences have clinical relevance, and data can be compared with greater precision are missing. Additionally, there should be a greater homogenization in experimental designs in order to compare data.

On the other hand, more studies are needed to evaluate another type of irrigant to use between each activation, such as EDTA, even combining its use with NaOCl.

## CONCLUSION

There are no significant differences in the remaining area with  $\text{Ca}(\text{OH})_2$  in the apical third of curved canals when comparing removal through activated irrigation with sonic versus ultrasonic device using SEM.

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### **Activación sónica versus ultrasónica en remoción de hidróxido de calcio en conductos curvos: estudio in-vitro.**

**Resumen:** Objetivo: Comparar el área de hidróxido de calcio (Ca(OH)<sub>2</sub>) remanente en el tercio apical de conductos curvos, tras activación sónica y ultrasónica, utilizando Microscopio Electrónico de Barrido (MEB). Metodología: Se recolectaron molares mandibulares permanentes. Se incluyeron los que en la raíz mesial presentaron radiográficamente una curvatura entre 15° y 30°. Se instrumentaron 39 conductos mesiolinguales con ProTaper rotatorio y se rellenaron con Ca(OH)<sub>2</sub> mezclado con propilenglicol. Se dividieron aleatoriamente en 3 grupos de acuerdo al tipo de remoción. Grupo I: tres inserciones con lima F2 irrigando con 2 mL de hipoclorito de sodio (NaOCl) 5.25% entre cada inserción. Grupo II: tres activaciones sónicas, irrigando con 2 mL NaOCl 5.25% entre activaciones. Grupo III: tres activaciones Ultrasónicas irrigando con 2 mL

NaOCl 5.25% entre activaciones. Se dejaron 3 conductos sin rellenar y otros 3 con relleno total como controles positivo y negativo respectivamente. Luego se fracturaron longitudinalmente obteniendo 2 secciones, se microfotografió el tercio apical del conducto radicular de cada sección con MEB y posteriormente en un área de 100.000 um<sup>2</sup>, se midió la superficie con Ca(OH)<sub>2</sub> remanente de ambas secciones usando ImageJ 1.47. Se analizó con ANOVA de una vía usando GraphPad-Prism 5.01. Resultados: El porcentaje de área con Ca(OH)<sub>2</sub> remanente del grupo I fue 62.93%. Grupo II, 51.77%. Grupo III 58.90%. No hubo diferencia significativa entre los tres grupos (P>0.01). Conclusión: No hay diferencias significativas en el porcentaje de área con Ca(OH)<sub>2</sub> remanente del tercio apical de conductos curvos, al comparar la activación sónica con la ultrasónica, utilizando MEB.

**Palabras clave:** *Endodoncia, hidróxido de calcio, remoción, sónico, ultrasónico.*

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