Calcium-Ion Diffusion of Four Calcium-Hydroxide-Based Materials: Ultralac XS, Vitapex, Roeko Calcium-Hydroxide Plus Points, and Pure Calcium Hydroxide Through Radicular Dentin

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Abstract
The aim of this study was to determine the amount of Ca diffusion through radicular dentin from 4 calcium-hydroxide-based materials placed in root canals following different treatments. The materials studied were (U) Ultradent-Ultralac XS, (D) Diadent-Vitapex, (R) Roeko Calcium Hydroxide Plus points, and (M) Merck-pure calcium hydroxide. Ninety freshly extracted human teeth were decrowned to a standard length of 14 mm, instrumented, irrigated with 5.25% NaOCl and divided into 5 groups. Group 1 was divided into 4 subgroups that received one of the following treatments prior to obturation with U: (a) ultrasound, (b) 50% citric acid, (c) ultrasound and citric acid, and (d) no treatment. Groups 2, 3, and 4 were similarly treated with D, R and M, respectively. Group 5 acted as the control group and was divided into two subgroups: (a) biomechanical preparation only and (b) no preparation. Ca diffused (mg/dl) in water through dentin as a function of time (1, 3, 5 and 16d) and was measured by spectrophotometry. Ca diffusion (mean±SD) through dentin from different materials as a function of different treatments and time covered a wide range (6.37-35.99). For all groups and time intervals, the amount of Ca diffused from different materials was the following: (U) 27.75±14.28, (D) 21.42±12.20, (R) 14.55±12.54, and (M) 26.74±14.14. From day 1 to day 16, there was a steady release of Ca from U, D, and M but not from R. Ultrasound and citric acid treatment yielded the highest (significant at p<0.0001) Ca diffusion (40.57±18.79) with R, followed by M (34.82±27.99), D (20.18±6.66) and U (18.32±6.21) at day 1. The highest diffusion of Ca ions for all materials occurred after the use of ultrasonic cavitation with citric acid irrigation. This study found Ca diffusion through dentin is influenced both by the nature of the material and type of treatment.

Keywords:
calcium hydroxide, calcium-ion diffusion, spectrophotometry

Introduction
The properties of calcium hydroxide have established it for three decades as the most widely used material for endodontic therapy. For this reason, new calcium-hydroxide-based materials continue to be introduced into the market while investigators search for higher calcium-ion and hydroxyl-ion release from the calcium-hydroxide molecule.

Therefore, we need to learn which biomaterial releases more calcium and hydroxyl ions through the radicular dentin and thus provides a higher clinical efficiency. It is important to understand the calcium-ion release of new calcium-hydroxide-based materials through radicular dentin and to determine if the new calcium-hydroxide products are better than the chemicals already in the market. This understanding can help to predict the success of different endodontic treatments requiring intracanal calcium
-hydroxide medication. Since its introduction (1) calcium hydroxide has been recommended as the best intracanal medication for a number of clinical scenarios. Several biological properties have been attributed to it, such as antimicrobial activity (2-11) antifungal activity (12, 13) the ability to dissolve tissues (14-16) the inhibition of radicular resorption (17) biocompatibility with induction of formation of hard tissue (18, 19) and an anti-inflammatory effect.

Therapeutic effects of calcium hydroxide derive from the constant release of calcium ions and hydroxyl ions (20). The beneficial effects are attributed to the alkaline properties of hydroxyl during its diffusion through dentin and also to the effects of the calcium ion in the formation of mineralized tissue (21). Reliable alternatives for the measurement of the calcium ion are the atomic absorption method, the hydroxyproline method, and the direct colorimetric method (22-29).

Calcium hydroxide in different presentations has been recommended as intracanal medication to stop reabsorptive processes if the pulp of traumatized teeth has been affected irreversibly and the therapy of root canals is suitable to stop the inflammatory process (17). Even though the treatment is effective, the action mechanism of calcium hydroxide is not fully understood.

Common calcium-hydroxide products are delivered as semi-solid pastes, gels, adhesives or cements, and some investigators suggest adding other substances to provide it with antibacterial action, fluidity, and consistency (30). In recent years calcium-hydroxide-based materials, such as CRCS, Sealapex, Ultracal XS and Roeko gutta-percha cones that contain calcium hydroxide, have been developed with biological capacities and biocompatibility and the ability to create a more favorable healing environment (alkaline pH) (31–33).

The endodontics principle of “what is removed from the canal is more important than what is introduced” indicates that all organic and inorganic contents of the canal must be removed. This principle has been supported in the past (34, 35) citric acid performed better than sodium hypochlorite at 5.25% in cleaning the root canal in conventional endodontics treatment.

In 1975, citric acid was first introduced in endodontics to clean root canals after pulpectomy, and scanning electron microscope revealed that it gave rise to clean surfaces (without organic and inorganic debris) (36). In addition, compared to other cleaning agents, citric acid improved tissue penetration and adherence to dentinal walls of the cementing material. No contraindications were observed in clinical practice in more than 1000 patients, and 50% citric acid had results similar to those of stronger acids (22, 36).

Therefore, the following are the aims of this research project:

1. The general objective of this study is to determine the diffusion capability through the radicular dentin of the calcium ion from four calcium-hydroxide-based materials.

2. The specific objective is to determine the quantity of the calcium ion liberated by the experimental biomaterial by means of four different treatments.

**Hypothesis**

The hypothesis states that differences exist in the quantity of calcium ions liberated in each of the four biomaterials. If this is true, it may be due to differences in the formulation of each obturation material. This variety of composition, a variable that must be considered, determines the mechanism and effectiveness of the product. This in-vitro study will also evaluate if different methods of root canal treatment affect Ca-ion diffusion.

**Materials and Methods**

Ultracal XS (Ultradent Products Inc.) is a radiopaque paste consisting of 41% distilled water, 35% calcium hydroxide, 19% barium sulfate, 3% propylene glycol, and 2% methil celulusa. It is designed for easy, controlled dispensing for a lining or as a temporary dressing and is available as a watery paste of calcium hydroxide contained in a 1.2 ml syringe with a pH of 12.5.
The Roeko Calcium Hydroxide Plus cone (Coltene/Whaledent/Roeko, Langenau, Germany) slowly releases calcium hydroxide from a gutta-percha matrix. It is a compound of 52% calcium hydroxide, 42% gutta-percha, sodium chloride, surfactant, and color agents. Sodium chloride and the surfactant improve the solubility of calcium hydroxide and the mobility of the ions. The cone is flexible for easy application and is standardized according to ISO.

Vitatapex (NEO Dental Chemical Products Co., Tokyo, Japan), which is distributed in North America by DiaDent, Canada, contains 40.4% iodoform, 30.3% calcium hydroxide, 22.4% silicone oil, and 6.9% inert material. Iodoform, a known bactericide, is released from the sealer to suppress bacteria in the canal or periapex.

Ninety (90) extracted single-rooted human teeth were divided into 1 control group of 10 teeth and 4 experimental groups of 20 teeth each, according to the type of material used and subdivided according to the treatment performed. The variables taken into consideration were the type of material, the diffusion degree of the calcium-ion, the type of treatment, and the observation time.

The teeth were collected and placed in formalin at 10%. They were later washed with sodium hypochlorite at 5.25% to prevent any contamination whatsoever. Once the crown was removed, a standardized length of 14 mm was established. Next, root surfaces were scaled to remove the cementum. Root canals were then submitted to biomechanical preparation with the Crown-Down technique and rotary instrumentation with ProTaper rotary instruments (Dentsply, Tulsa Dental, Tulsa OK). Specimens were divided into 4 groups of 20 teeth each and a fifth group, the control group of 10 teeth.

**Group 1:** 20 specimens obturated with Vitapex were subdivided in 4 groups of 5 teeth each:
1(a) 5 specimens with ultrasonic filing (U.S.) as the last step of the biomechanical preparation,
1(b) 5 specimens with final irrigation using citric acid at 50% (C.A.),
1(c) 5 specimens with ultrasonic filing as the last step of biomechanical preparation and their canals soaked with citric acid at 50% (C.A.+U.S.),
1(d) 5 specimens with the rotary biomechanical preparation only, without final ultrasonic instrumentation and/or citric acid irrigation (None).

**Group 2:** 20 specimens obturated with Ultracal XS were subdivided in the same 4 groups with 5 specimens each as described for group No. 1.

**Group 3:** 20 specimens obturated with Roeko Calcium-Hydroxide Plus Points were subdivided in the same 4 groups with 5 specimens each as described for Group 1.

**Group 4:** 20 teeth obturated with pure calcium-hydroxide were subdivided in the same 4 groups of 5 specimens each as described for Group 1.

**Group 5:** 10 teeth acted as controls: 5 were subject to biomechanical preparation only, and 5 were not prepared at all.

Later, coronal and apical accesses were filled with sticky wax, and, to guarantee proper sealing, a double coat of nail polish was applied. Once the preparation of each sample was completed, they were each placed in a test tube with 2 ml of deionized water in order to measure the calcium-ion concentration while in solution, using the colorimetric spectrophotometric method (Milton Roy Spectronic 601, Spectronics Instruments, Inc. Rochester NY). The Ca–ion diffusion of each specimen was measured on day 1 (day 0–day 1), day 3 (day1–day 3), day 5 (day 3–day 5), and day 16 (day 5–day 16).

The specimens remained in an incubator at 37°C to simulate the temperature of the oral cavity without the presence of carbon dioxide. At 24 hours the procedure of absorbance reading began; the materials and reagents were prepared under sterile conditions in the Laminar Flow Clean Air Workstation. Then the reagent was prepared following the instructions indicated by the manufacturer (Calcium Liguicor, Colorimetric test, monoreagent, o-cresolphthalein–complexone (CPC) method, Human GmbH, Wiesbaden, Germany) mixing volumes of the buffer solution and the liguicor reagent in adequate quantities for the specimens. The total volume of the homogenized mixture equaled 1 ml. This mixture
reached ambient temperature in 10 minutes and was then poured into Eppendorf 0.2 ml PCR tubes for each specimen to be analyzed.

During each duration period, a sample of 20 microliters from the standard solution was obtained from each specimen. Two Eppendorf tubes were obtained: one tube was the positive control with 1 ml of the reagent plus 20 microliters of the standard, and one tube had 1 ml of the reagent alone. The remaining standard solution was disposed of and then replaced with 2 ml of deionized water.

The optic density of the specimens were then measured by spectrophotometry to 570 nm (lamp of tungsten) adjusting the target to zero absorbance with 1 ml of the work reagent. For the calculation of the concentration of the calcium, a formula was applied (C : 8mg/dl = 8×absorbance 570 sample/absorbance 570 standards).

**Tabulation and Statistical Analysis**

Initially, concentrations for the diffusion of the calcium ion were tabulated according to the time of observation, type of material, and applied treatments. Three-factor analysis of variance (ANOVA) with factors for time, treatment, and material, and their interactions was initially employed to test for differences in calcium-ion concentrations. Since significant interactions existed between the main factors, the data were ultimately analyzed as a series of one-way classifications. Tukey’s HSD multiple comparison procedure was used as the post-hoc mean separation test.

**Results**

The average concentration of calcium for each material at all four time increments (Days 1, 3, 5, and 16) is illustrated in Fig. 1. Three of the materials showed an increase in the diffusion rate progressing from day 1 to day 16: pure Ca(OH)_2, Ultracalc XS NF, and Vitapex. However, for the Ca(OH)_2 gutta-percha points, the average rate decreased.

For pure calcium hydroxide, the calcium-ion diffusion averaged 26.74 mg/dl, releasing the most calcium-ions on day 16 (31.35 mg/dl). The largest calcium-ion diffusion occurred with ultrasound and citric acid : 34.82 mg/dl on day 1.

For Ultracalc XS, the average calcium-ion diffusion after 16 days was 27.75 mg/dl, and the largest ion release was on day 16 (31.65 mg/dl). Calcium-ion release on day 1 was 19.35 mg/dl, and on day 3 it was 28.63 mg/dl. Throughout the study, this was the largest ion release among the different materials (27.75 mg/dl) (Table 1).

For Vitapex, the average calcium ion diffusion for the 16 days was 21.42 mg/dl, releasing the most calcium ions on day 16 (26.33 mg/dl). Among the various treatments, the higher diffusion was with ultrasound and citric acid : 20.18 mg/dl on day 1.

For the Roeko Calcium–Hydroxide Plus points, diffusion average during the 16 days was 14.55 mg/dl. More calcium ions were released on day 1 (15.33 mg/dl). As Fig. 2. illustrates with treatment, the highest diffusion rate was with ultrasound and citric acid : 40.57 mg/dl on day 1.

In the past, the rate of ion diffusion for Ca(OH)_2 gutta-percha points measured lower than the other Ca(OH)_2 releasing materials. This study found that by using the combination of ultrasonic cavitation
and citric-acid irrigation, high rates of diffusion from the cones can be achieved. Table 3 (day 1) shows how the Ca(OH)_2 releasing cones outperformed all other materials, which were under the same conditions. This is reflected in Fig. 3, which displays the significance of combining treatment with material. The greatest significance was in combining ultrasound cavitation and citric-acid irrigation with the placement of the Roeko Ca(OH)_2 gutta-percha points.

**Discussion**

A major point that this study revealed is the importance of post-preparation treatment. Table 2 (day 16) shows that combining ultrasonic filing and citric-acid irrigation effectively increased ion diffusion in all four materials tested.

Antimicrobial activity of calcium hydroxide depends on hydroxyl-ion release in an aqueous environment. Hydroxyl-ions are oxidizing free radicals that are highly reactive and react to several biomolecules (3).

In a previous study, calcium-ion concentration was determined by placing the teeth in an aqueous environment, where the measurements were determined in three dissolution stages: dissolution, dissolution and diffusion I, dissolution and diffusion II (21). Results showed that calcium-ion diffusion was observed in the first 16 days in all of the instances in which pure Ca(OH)_2 was applied.

The use of ultrasound plus an irrigant such as sodium hypochlorite has long been recommended (37). Within 3 to 5 minutes of irrigation, canal walls were free of smear layer. This study found that the combined treatment of ultrasonic cavitation and irrigation with citric acid resulted in the largest diffusion of the calcium ion. However, a comparison between the efficiency of ultrasonic cleaning and manual instrumentation with sodium hypochlorite at 2.5% or water and SEM revealed that ultrasonic cavitation does not play an important role in the debridement of the root canal system (38).

Citric acid is an effective irrigant of the root canal when it is used alternately with sodium hypochlorite (36, 39, 40). Citric acid in 10%, 25%, and 50% concentrations cleans the walls of root canals leaving the dentinal tubuli open, but the NaCl solution at 5.25% blocked the dentinal tubuli when it was used as the single irrigant in the root canal preparation (41). In an evaluation of the antimicrobial effectiveness of
Table 3. Average concentration of Ca (mg/dl) on day 1 for each material according to the treatment

<table>
<thead>
<tr>
<th>Post-preparation Treatment</th>
<th>MATERIALS</th>
<th>Roeko Calcium Hydroxide Plus Points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure Ca(OH)₂</td>
<td>Ultrcal XS</td>
</tr>
<tr>
<td>C.A.</td>
<td>24.32±9.70</td>
<td>28.79±7.68</td>
</tr>
<tr>
<td>None</td>
<td>6.40±4.14</td>
<td>5.70±4.00</td>
</tr>
<tr>
<td>U.S. + C.A.</td>
<td>34.82±27.99</td>
<td>18.32±6.21</td>
</tr>
</tbody>
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Fig. 3. Significance resulting from the combination of obturation material and post-biomechanical treatment. Significance represents the degree of impact made on the diffusion capability of each material by utilizing the different approaches to treatment.

citric acid as an irrigant of the root canal system (42), the germicidal effects of citric acid at 25% and 50%, sodium hypochlorite, and saline solution were compared at 5- and 15-minute intervals. NaOCl at 5.25% was more effective as an antimicrobial agent.

Among the materials analyzed in this study, Ultrcal XS showed the largest diffusion average. This is in agreement with previous findings (43) indicating that Ultrcal XS presented more calcium–ion diffusion through dentinal tubuli compared to Calasep, Roeko calcium–hydroxide–releasing points, and pure calcium hydroxide although the differences were not statistically significant.

The main advantage of using Vitapex calcium hydroxide with iodoform in primary teeth is that this paste reabsorbs from the apical tissues within a week to two months, and apparently it does not damage the permanent tooth (44, 45). These findings agree with this research because the largest calcium release for Vitapex was on days 5 and 16.

In an in-vitro study (46) comparing calcium–hydroxide–releasing gutta-percha cones, pure calcium–hydroxide powder mixed with distilled water, and Reogan Rapid (Vivadent Ets, Schaan, Liechtenstein), which is a non-setting calcium–hydroxide preparation, the calcium–hydroxide releasing points showed a lower alkalizing potential, and the ion diffusion rate was lower than that of Reogan Rapid and of calcium–hydroxide mixed with distilled water (46). Our findings agree with the previous studies in which calcium–hydroxide–releasing points released less calcium hydroxide than the other materials. This difference may be due to gutta–percha matrix slowing the release. The cones, however, still are effective for specific clinical situations (47), such as emergency treatment, treatment for root resorption, and especially as a temporary filling. An in-vitro study that compared various medicament–embedded gutta–percha cones showed that calcium–hydroxide demonstrated superior reduction or elimination of bacterial growth (48).

Nevertheless, Ca(OH)₂ cones offer unique advantages to the clinician. These cones, which are ready to use, do not smear during insertion and do not leave residue upon removal. They are very easy to apply and remove. Since they can be inserted down to the apex, calcium–hydroxide is released throughout the entire canal.

Conclusions

Calcium–ion release steadily increased from day 1 to day 16 for pure Ca(OH)₂, Ultrcal XS, and Vitapex. Ultrcal XS gave the highest overall diffusion rate. Pure Ca(OH)₂ did give results that were relatively close; however, Vitapex diffused at an even lower rate. Roeko Ca(OH)₂ points gave the lowest overall ion diffusion rates.

There was a statistically significant difference between the calcium–ion diffusion of the different
materials that received different treatments that were established for calcium release through radicular dentin. We discovered that the treatment of the canal before placing the calcium-hydroxide material affects the diffusion of ions. The highest diffusion of Ca+ ions occurred after the use of ultrasonic cavitation with citric-acid irrigation.

The largest calcium-ion release occurred during the first 24 hours with ultrasonic filing and citric acid at 50% using Roeko Ca(OH)\textsubscript{2} cones (p<0.0001) This was the only time that these cones outperformed all the other tested materials.

In addition to calcium-ion diffusion, Roeko points also offer other advantages. Once these medicament-embedded gutta-percha cones are inserted, Ca+ ions are distributed evenly throughout the canal. Upon removal, no residue of calcium hydroxide remains on the canal wall.

References