

A titration model for evaluating calcium hydroxide removal techniques

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ABSTRACT

Objective: Calcium hydroxide ($\text{Ca}(\text{OH})_2$) has been used in endodontics as an intracanal medicament due to its antimicrobial effects and its ability to inactivate bacterial endotoxin. The inability to totally remove this intracanal medicament from the root canal system, however, may interfere with the setting of eugenol-based sealers or inhibit bonding of resin to dentin, thus presenting clinical challenges with endodontic treatment. This study used a chemical titration method to measure residual $\text{Ca}(\text{OH})_2$ left after different endodontic irrigation methods. **Material and Methods:** Eighty-six human canine roots were prepared for obturation. Thirty teeth were filled with known but different amounts of $\text{Ca}(\text{OH})_2$ for 7 days, which were dissolved out and titrated to quantitate the residual $\text{Ca}(\text{OH})_2$ recovered from each root to produce a standard curve. Forty-eight of the remaining teeth were filled with equal amounts of $\text{Ca}(\text{OH})_2$ followed by gross $\text{Ca}(\text{OH})_2$ removal using hand files and randomized treatment of either: 1) Syringe irrigation; 2) Syringe irrigation with use of an apical file; 3) Syringe irrigation with added 30 s of passive ultrasonic irrigation (PUI), or 4) Syringe irrigation with apical file and PUI ($n=12/\text{group}$). Residual $\text{Ca}(\text{OH})_2$ was dissolved with glycerin and titrated to measure residual $\text{Ca}(\text{OH})_2$ left in the root. **Results:** No method completely removed all residual $\text{Ca}(\text{OH})_2$. The addition of 30 s PUI with or without apical file use removed $\text{Ca}(\text{OH})_2$ significantly better than irrigation alone. **Conclusions:** This technique allowed quantification of residual $\text{Ca}(\text{OH})_2$. The use of PUI (with or without apical file) resulted in significantly lower $\text{Ca}(\text{OH})_2$ residue compared to irrigation alone.

Keywords: Calcium hydroxide. Ultrasonic therapy. Glycerin. Therapeutic irrigation.

INTRODUCTION

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) as an intracanal medicament (ICM) has been extensively studied and its clinical use well established^{3,17,23-26}. In aqueous solution $\text{Ca}(\text{OH})_2$ dissociates into calcium and hydroxyl ions. The large amount of hydroxyl ions liberated interferes with the bacterial cytoplasmic membrane integrity, largely by interruption of transfer of nutrients and destruction of phospholipids from unsaturated fatty acids¹⁰.

In vitro studies have demonstrated potential clinical concerns regarding the inability to fully remove calcium hydroxide. Residual $\text{Ca}(\text{OH})_2$ may interfere with sealer entrance into dentinal tubules and inhibit bonding of resin to dentin⁶. Additionally, leakage may be increased with the use of calcium

hydroxide as an ICM^{13,14} or residual $\text{Ca}(\text{OH})_2$ may interfere with the setting of eugenol based sealers or MTA^{19,27}.

A variety of $\text{Ca}(\text{OH})_2$ removal techniques have been studied. Irrigation-only techniques appear to result in poor $\text{Ca}(\text{OH})_2$ removal¹⁵, while use of a master apical file or passive ultrasonic irrigation (PUI) for $\text{Ca}(\text{OH})_2$ removal have been found efficacious^{12,22,28}. For review of PUI, see van der Sluise, et al.²⁹ (2007). Quantification of residual $\text{Ca}(\text{OH})_2$ remaining in the root has been attempted by using digital images^{4,8,12,22}, while nonparametric grading systems have been used with digital photographic images in teeth with premade grooves^{11,30}. Concerns exist, however, regarding two-dimensional quantification on a nonplanar surface and the inability to differentiate

debris vs. $\text{Ca}(\text{OH})_2$. More recently, spiral CT²⁰ and micro CT have been used to study calcium hydroxide removal²¹. These studies are all problematic in that they cannot accurately detect residual $\text{Ca}(\text{OH})_2$ in actual teeth.

The technique proposed by Bramante allows reuse of specimens, and thus reduces inaccuracies due to different specimen anatomy⁵. With reuse of the same specimens, however, cumulative effects of the chelating agent EDTA can occur and removal of residual $\text{Ca}(\text{OH})_2$ cannot be confirmed. Teeth used in this study had a standardized canal preparation and were only used once.

Visual identification of residual $\text{Ca}(\text{OH})_2$, even with SEM, is not accurate. Since $\text{Ca}(\text{OH})_2$ has a high pH (pH>12), this attribute may be used to identify residual $\text{Ca}(\text{OH})_2$ left in the root canal system by pH determination of known amounts retrieved from the canal compared to unknown amounts in canals after various irrigation methods have been used. The purpose of this study was to employ a chemical microtitration technique to test removal methods of $\text{Ca}(\text{OH})_2$ paste (Calasept®; JB Dental, Ridgefield, Connecticut, USA) from the root canal system.

MATERIAL AND METHODS

This study was exempted by the Institutional Review Board of the University. Eighty-six extracted single canal maxillary and mandibular canines were stored in normal saline with 0.2% sodium azide. Samples were randomized into groups. Three teeth served as positive controls and three as negative controls. Thirty teeth served as standards.

Preparation of specimens

Teeth were decoronated at the cemento-enamel junction and radiographed from the proximal and buccal view. The Pruett, Clement, and Carnes method was used to standardize curvature at $\leq 15^\circ$ ²¹. Two samples were excluded due to aberrant anatomy. Root length was standardized at 17.5 mm. A glide path was established to a #25 Flex-O hand file (Dentsply-Maillefer, Johnson City, Tennessee, USA) and K3 nitinol files (SybronEndo, Cuyahoga Falls, Ohio, USA) were used to prepare each tooth according to manufacturers' recommendations to a 50/0.06 final apical file (FAF). Patency was established with size 20 Flex-O file. Irrigation with 1 mL of NaOCl 5.25% and recapitulation was performed between files. The length of the Max-i-probe (Dentsply, Elgin, Illinois, USA) was set at 2 mm from the working length (WL). Thirty seconds of passive ultrasonic irrigation with 5.25% NaOCl was performed with a one minute soak using 17% EDTA to remove smear layer. A final 3 mL rinse of 5.2% NaOCl was performed and all canals were dried with paper points. Canals were then filled with Calasept®

[$\text{Ca}(\text{OH})_2$ based ICM] by inserting the syringe tip until locked in, then loosening and backfilling. Radiographs confirmed a dense $\text{Ca}(\text{OH})_2$ fill. Teeth were temporized with Fuji IX resin modified glass ionomer (GC Corporation, Tokyo, Japan) and placed in an incubator (Precision Scientific, Chennai, India) at 37°C for one week in a humid environment.

Standards

In order to develop a standard curve using known amounts of $\text{Ca}(\text{OH})_2$, thirty teeth were selected. The identical radiographic and the preparation protocol were utilized for the standard teeth and for unknown samples, except that different known weights of $\text{Ca}(\text{OH})_2$ were added to the teeth (much smaller weights to replicate residual $\text{Ca}(\text{OH})_2$ after irrigation methods). After temporization and 7 days of incubation, the glycerin transfer and titration steps were performed in identical fashion to samples. A standard curve was generated by graphing the different known weights added to the teeth *versus* the pH recording after addition of known micromoles of HCL. Samples were titrated using 0.025, 0.05, 0.1, 0.5, 1, or 3 M HCL with 10 microliter aliquots using a micropipette. Usually titrations began with 10 microliters of 0.1 M. If the initial pH was higher, the operator may have used a higher molarity such as 0.2 M. If the initial pH of the mixture was lower (i.e. 11.0-11.4), the operator may have begun with a weaker concentration (i.e. 0.05 M HCL). pH measurements were recorded after each addition of HCL using a model HO4N-0001 micro pH electrode (Lazar Research Laboratories, Los Angeles, California, USA) and a Model 60 pH meter (Lazar Research Laboratories, Los Angeles, California, USA). After each aliquot addition of acid to the microcentrifuge tube, the tube was vortexed for 10 s. Adequate time was given for each pH measurement – this was approximately 10-60 s for the meter to equalize.

After creating a titration curve for each sample, linear regression was applied to each curve. The neutral point, pH=7, was selected for use in each regression curve. By solving for pH=7, cumulative micromoles of HCL added could be determined for each titration.

A second standard curve was calculated using only $\text{Ca}(\text{OH})_2$ in microcentrifuge tubes and not placed in teeth. In the second standard curve, small amounts of Calasept® was added to preweighed empty microcentrifuge tubes. Eleven different $\text{Ca}(\text{OH})_2$ weights were made in triplicate and used to produce the standard curve. 100 microliters of 60% glycerin at 40°C was added to each microcentrifuge tube and vortexed for 30 s to ensure full dissolution of the small amount of $\text{Ca}(\text{OH})_2$, with HCL titration and pH measurements completed.

Treatment groups

Samples were removed from incubator and temporary fillings removed. For each group (48 teeth; n=12/group), a #30 Flex-O file and a #50 Flex-O file were used for gross removal of Calasept® before each irrigation technique: Group 1: Irrigation (NaOCl 5.2% 3 mL followed by EDTA 17% 3 mL. A final rinse of NaOCl 5.2% 5 mL was performed). Group 2: Irrigation (as in group 1) with the addition of a K3 #50-0.06 taper instrumented to WL between the first two rinses. Group 3: Irrigation (as in group 1) with the use of PUI for 30 s between the first NaOCl and EDTA rinse. Group 4: consisted of NaOCl 5.2% 1.5 mL, use of a K3 #50-0.06 instrumented to WL, 1.5 mL NaOCl, 30 s of PUI, and the final EDTA and NaOCl rinses as in previous groups.

Three teeth were selected for negative controls. These teeth were instrumented but canals were left empty. Negative control teeth were included in the experimental sample set and operator was also blinded to control teeth. Positive controls consisted of three microcentrifuge tubes with saturated solutions of $\text{Ca}(\text{OH})_2$.

Removal of residual calcium hydroxide

Any residual $\text{Ca}(\text{OH})_2$ remaining in the tooth after gross $\text{Ca}(\text{OH})_2$ removal was removed by the following manner: a preparation of 60% glycerin (Humco Corporation, Texarkana, TX): 40% distilled water at 40°C was placed into the canal with a Ultradent capillary tip (Ultradent Products, Inc., South Jordan, Utah, USA). PUI for 10 s was performed with a #15 Zipperer file (Roydent, Rochester Hills, Minnesota, USA) at 2 mm from

WL to help the remaining Calasept® dissolve. The glycerin with dissolved $\text{Ca}(\text{OH})_2$ was removed using a narrow Ultradent tip and a 10 mL syringe, with the aliquots placed into a 1.5 mL microcentrifuge tube (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Aliquots were repeated until 100 microliters was obtained.

A single calibrated 20 microliter Pipetman micropipette (Gilson Inc., Middleton, Wisconsin, USA) was used for all titrations. Each microcentrifuge tube was labeled with a second random number to blind the operator. A titration curve was generated by adding 10 microliter aliquots of 0.025, 0.05, 0.1, 0.5, 1, or 3 M HCL using a micropipette. To ensure a good mix, each microcentrifuge tube was vortexed with a Vortex Genie Mixer (Scientific Products, Evanston, Illinois, USA) for 10 s between additions and heated to $40^\circ\text{C} \pm 1^\circ$ in a Hanau® low temperature water bath (Teledyne Water Pick, Fort Collins, Colorado, USA). pH measurements were recorded after each addition of HCL using a model HO4N-0001 semi-micro pH electrode (Lazar Research Laboratories, Los Angeles, California, USA) and a Model 60 pH meter (Lazar Research Laboratories, Los Angeles, California, USA). Based on pilot studies, an algorithm describing which molarity to add based on the current pH was made. The micro pH electrode and meter were calibrated with standard pH solutions (Omega Scientific, Tarzana, California, USA). After each pH measurement, the tip of the electrode probe was thoroughly rinsed with distilled water and wiped with a Kim Wipe® (Kimberley-Clark Professional, Mississauga, Ontario, CA). All pH readings were

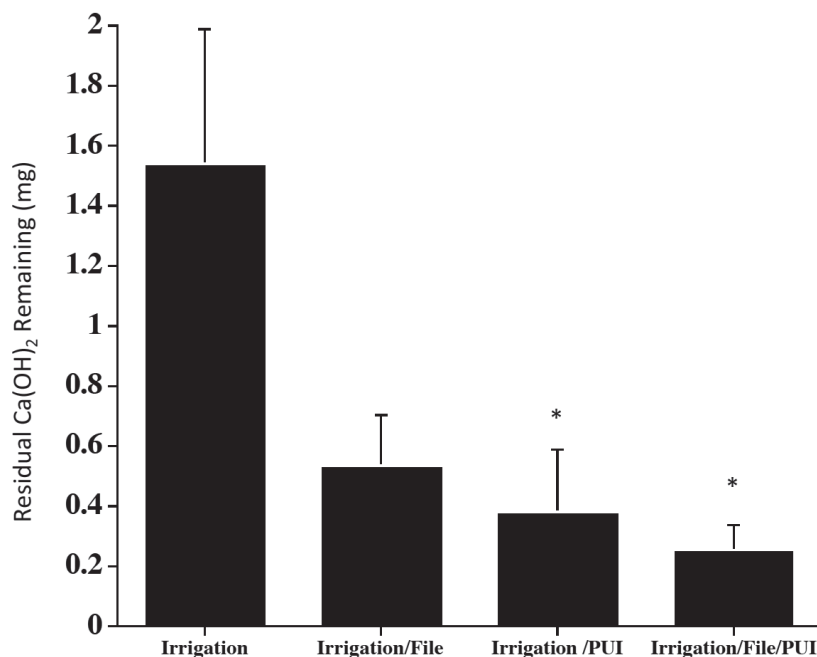


Figure 1- Residual calcium hydroxide remaining after each removal technique (means±S.E.M.).* p<0.05 compared to “Irrigation Only” group. PUI=passive ultrasonic irrigation

recorded. The chemical reaction between Ca(OH)_2 and HCL is described by the equation: $2 \text{HCl} + \text{Ca(OH)}_2 \rightarrow \text{CaCl}_2 + 2\text{H}_2\text{O}$.

Standard deviations and means were calculated for each group, and pairwise comparisons were made using a Tukey-Kramer multiple comparisons adjustment.

After the experiment, several representative teeth were split and SEM (Model TM3000 Table Top Microscope, Hitachi High-Technologies Corporation, Tokyo, Japan) was performed to visually assess the inside canal surface.

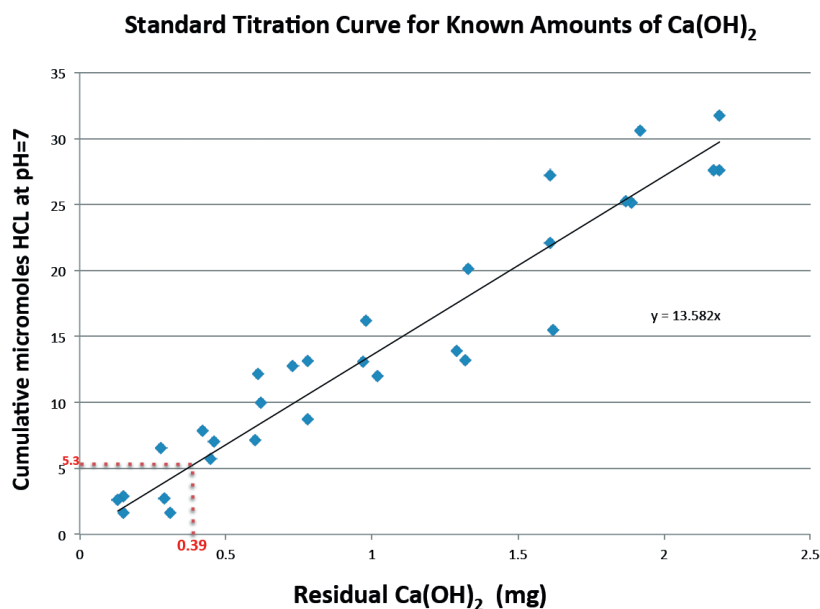


Figure 2- Sample standard curve. Cumulative μmoles of HCL at pH=7 for each standard was plotted against initial known weights (mg) of Ca(OH)_2 . For example, the vertical red dotted line indicates that 0.39 mg of Ca(OH)_2 standard would take 5.3 μmoles of HCL to reach pH=7.0 (horizontal red dotted line). Using these standard known amounts, we can create a linear regression line to determine the amounts of Ca(OH)_2 in our unknown samples by the μmoles of HCL used to get to pH=7.0

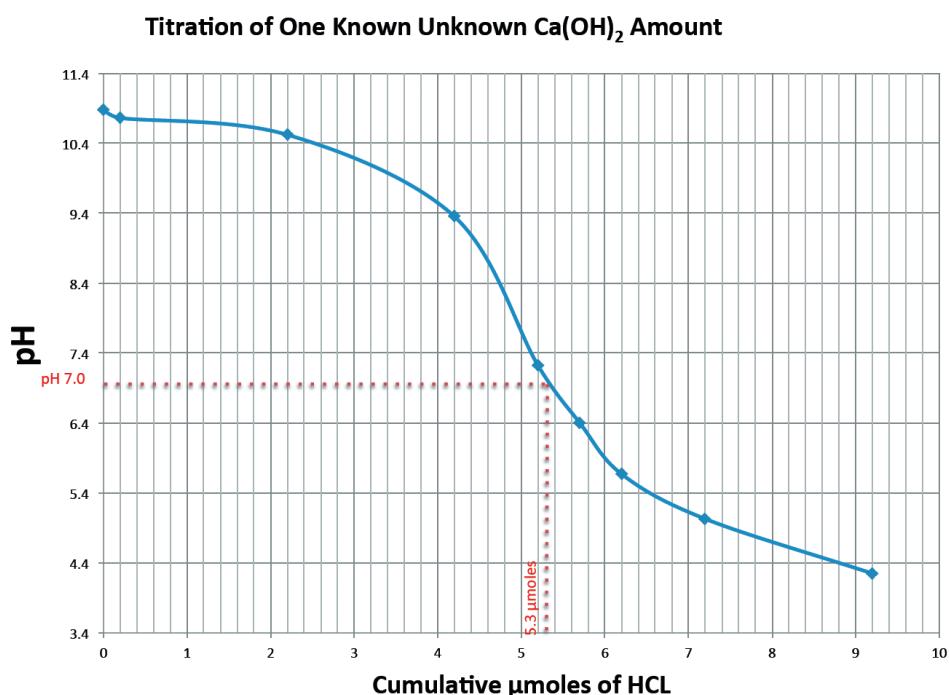


Figure 3-A titration curve was made from utilizing data points for an unknown sample titrated with HCL to pH 7.0. From the μmoles used at pH 7.0, we can determine the amount Ca(OH)_2 in our sample using the standard curve in Figure 2

RESULTS

Figure 1 shows the means and standard deviations of residual calcium hydroxide after the various removal group techniques were applied. The groups differed significantly [$F(3,42)=4.47$, $p=0.0082$], indicating that there was a difference between the group means. The group 1 (irrigation only) mean was significantly different than the means of groups 3 (PUI) and 4 (PUI + file), $p=0.0291$ and $p=0.0104$, respectively. No other comparisons were statistically significant. Negative controls [no $\text{Ca}(\text{OH})_2$ added] were found to have near neutral pH measurements, while positive controls [fully $\text{Ca}(\text{OH})_2$ saturated 100 μL of glycerin] required larger amounts of HCL to achieve neutrality.

A standard curve is illustrated in Figure 2. An example of a titration of one of the unknown samples and the corresponding linear regression line and equation is shown in Figure 3. The dotted red line shows that at pH 7.0, it took 5.3 μmoles

of HCL to neutralize this sample. Based on the standard curve of known samples in Figure 2, we can determine from the μmoles of HCL exactly how much $\text{Ca}(\text{OH})_2$ was present in the sample [in this case using the $y=13.582x$ linear regression formula, we now know $y=5.3$ and can solve for x as 0.39 mg of $\text{Ca}(\text{OH})_2$ in the unknown sample].

Examination of SEM's taken after our study revealed some debris mixed in with the glycerin (see Figure 4).

DISCUSSION

This study found agreement with previous studies that no $\text{Ca}(\text{OH})_2$ removal technique successfully removed all the calcium hydroxide from the canal system^{12,16,20,28}. This may pose a clinical problem since residual calcium hydroxide interacts with eugenol in ZOE based sealers, leading to residual eugenol in the set product¹⁹. Thus $\text{Ca}(\text{OH})_2$ may interfere with the obturation seal. The clinical implication of unset sealer is unclear and further clinical studies are needed to elucidate the effect of this interaction. Examination of SEMs taken after our study revealed some debris mixed in with the glycerin in the open canal. Perhaps the glycerin transfer failed to remove all the Calasept®, or the glycerin transfer *did* remove all the Calasept®, and what was seen mixed in with the remaining glycerin was actually dentinal debris. As part of the methods used in this experiment, collection of residual $\text{Ca}(\text{OH})_2$ included passive ultrasonic instrumentation between each transfer. This was done to ensure the $\text{Ca}(\text{OH})_2$ on the walls was incorporated into solution, and likely would have also incorporated more debris into the glycerin. One method to differentiate residual debris from Calasept® would be to radiolabel Calasept® and apply removal studies, which was not performed in this study. Then an autoradiographic analysis could be performed to determine "what we are actually looking at" after calcium hydroxide removal methods. Allison, et al.¹ (1979) used such a technique with ⁴⁵Ca to prove that step back preparations had less leakage than serial preparation. It appears that some $\text{Ca}(\text{OH})_2$ is left in the canal during most irrigation methods, mainly in the dentinal tubules, but amounts of residual $\text{Ca}(\text{OH})_2$ in the open canal could be minimized using passive ultrasonic instrumentation. Removal of medicament trapped within the dentinal tubules was attempted with repeated dissolution using glycerin extracts and passive ultrasonic irrigation in the same root, but could not be removed. The results here are for comparative purposes, and demonstrate that even with ideal conditions and access (decoronated tooth, straight root), medicament still remains in the canal. This may interfere with the setting of eugenol based

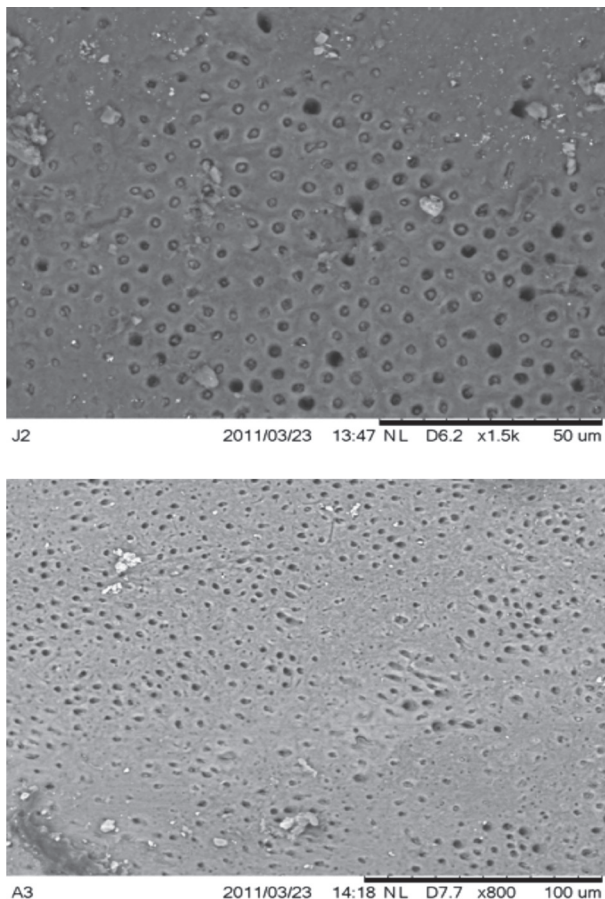


Figure 4- After the irrigation experiment, several teeth were split and scanning electron microscopy (SEM) (Model TM3000 Table Top Microscope, Hitachi High-Technologies Corporation, Tokyo, Japan) was performed to visually access the tooth surface. Representative micrographs of the apical one-third of different roots are shown at 1,500X (top) or 800X (bottom)

sealers or MTA^{19,27}.

One primary result of our study was that the 30 s use PUI (groups 3 and 4) produced better Ca(OH)₂ removal than the irrigation-only group. This is in agreement with many studies supporting the effectiveness of PUI^{4,9,12,30,31}. However, Lev, et al.¹⁸ (1987) found contrasting results in that 1 minute of passive ultrasonic step back was not significantly better in debris removal than irrigation and hand filing alone. The 30 s time frame was adequate to achieve statistical significance. This is in contrast to a study that examined debris removal after a 3 minute PUI time *per* canals (12 minutes *per* molar)². Another study utilizing sequential micro-CT scans studied Ca(OH)₂ removal with PUI activation for 60 seconds *per* canal (4 minutes *per* molar)³¹. Our results demonstrated two minutes (30 s *per* canal) of PUI for a typical four canal molar is adequate to remove most residual Ca(OH)₂ from the root canal. This is in agreement with Sabins, et al.²² (2003) who found 30 s of PUI adequate to significantly reduce debris levels in the mesial canals of mandibular molars²².

The agreement of our titration model with previous pixel or voxel-quantification studies confirms the accuracy of this technique for evaluating residual Ca(OH)₂ in the canal. This is very significant in that to our knowledge chemical quantification of residual calcium hydroxide has never been performed. In contrast, pixel-quantification methods examine debris and Ca(OH)₂ and, while they share many advantages, they also have limitations. Some potential issues with pixel-quantification methods include concerns with reuse of teeth for repeated testing. Another concern is the need for very uniform canals – necessary so that teeth can be predictably split. One Ca(OH)₂ removal study used mesial mandibular molar canals for uniformity in canal morphology¹². While standard preparation of canals was a part of this study, this measurement technique does not strictly require uniform canals. A titration technique, such as this, might be useful to evaluate Ca(OH)₂ removal from the more variable-sized distal canal or C-shaped canals. In a clinical setting, non-uniform canals may benefit the most from PUI.

A titration-quantification method differs from a voxel or pixel quantification method in that the former expresses results with a single value. A pixel or voxel-quantification method can give results in terms of location of remaining debris or Ca(OH)₂, while a chemical quantification method gives a number for the entire canal. This may be a disadvantage for a titration technique in that it may be more important to define Ca(OH)₂ removal specifically in one area, such as the apical one-third. It is interesting to note a titration technique gives results in mg of remaining Ca(OH)₂. Most studies

express results in percent, rather than actual weight of Ca(OH)₂.

The titration technique is time consuming. Also, the Ca(OH)₂ studied must be soluble in glycerin (or another solvent that can be titrated). Pilot studies indicated that Ultracal (Optident Ltd, International Develop Centre, West Yorkshire, UK) did not dissolve in glycerin. Further studies testing other solvents will allow more premixed Ca(OH)₂ pastes to be studied with a chemical titration method.

CONCLUSION

This study tested a new approach to quantification of residual Ca(OH)₂ by chemical microtitration. This model is novel and appears to be accurate and reliable, with several potential advantages. Adjuncts to irrigation such as PUI for 30 s or the use of a final apical file were shown to improve Ca(OH)₂ removal from the open canal, within decoronated teeth with single straight canals, however the medicament was not fully removed from all teeth. Further studies utilizing the chemical titration method may help us understand residual Ca(OH)₂ removal from diverse tooth types such as C-shaped molars and wide oval shaped canals.

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