Removal Efficacy of Various Calcium Hydroxide/Chlorhexidine Medicaments from the Root Canal with the Aid of Biological Microscope

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Aim: An in vitro study was conducted to comparatively evaluate the two techniques – hand instrumentation and ultrasonic file irrigation for removal of various calcium hydroxide (Ca(OH)₂)/chlorhexidine containing medicaments from the root canal by using Biological microscope and Digital Camera. Materials and Methods: Sixty-four human single-rooted teeth with straight canals were used. Root canal preparation was performed using Pro-taper Universal for Hand use (Dentsply Maillefer, Ballaigues Switzerland). Teeth were randomly divided into three Groups A, B, and C of 21 teeth each, the remaining tooth remained untouched and served as a negative control. Teeth of Group A were filled with Ca(OH)₂ mixed with saline. Teeth of Group B were filled with Ca(OH)₂ mixed with 0.2% chlorhexidine gel. Teeth of Group C were filled with Ca(OH)₂ mixed with 0.12% chlorhexidine solution. Teeth were stored at 37°C and 100% relative humidity for 7 days. Then Ca(OH)₂ was removed using two techniques: hand instrumentation and ultrasonic file irrigation in both groups. Teeth were sectioned longitudinally and observed under Biological microscope and Digital Camera. Results were statistically analyzed with the Mann–Whitney and Kruskal–Wallis test. Results: The results showed a significant difference between hand instrumentation and ultrasonic file irrigation in cervical and middle third, but no statistic difference in apical third. Conclusion: None of the techniques used in this study removed the inter-appointment root canal medicaments completely.

Keywords: Biological microscope, calcium hydroxide, hand instrumentation, ultrasonic file irrigation

INTRODUCTION
Thorough instrumentation supported by irrigation reduces the number of microorganisms in the infected root canal.[1] A long-standing endodontic infection allows bacteria to propagate to the entire root canal system including the ramifications, isthmuses, apical deltas, and dentinal tubules. In these locations, bacteria may remain even after complete chemomechanical preparation of the root canal.[2,3] These remaining bacteria grow and multiply inside the root canal system if no intracanal dressing is used between the appointments. Thus, intracanal medication may be a valuable adjunct to chemomechanical preparation in the disinfection of the root canal system, reducing the endodontic microbiota and therefore favoring periapical tissue repair.[4]

To obtain a wide spectrum of antimicrobial activity pastes consisting of calcium hydroxide (Ca(OH)₂) mixed with 2% chlorhexidine solution[5] or gel[6,8] have been investigated. Without the components adversely affecting the solubility and activity of one another, their combination exhibits an additive or synergistic effect on some endodontal pathogens such as Peptostreptococcus micros and Streptococcus intermedius[9] or Enterococcus faecalis.[3]

It has been reported that residual Ca(OH)₂ on the root canal walls influences dentine bond strength[10] and the penetration of sealers into dentinal tubules[11] markedly compromising the quality of the seal provided by the root filling.[12] The remnants...
of Ca(OH)₂ could also react chemically with various sealers reducing their flow and working time.[13]

Thus, complete removal of various Ca(OH)₂ containing medicaments from the root canal before obturation is recommended. The intracanal medicament is usually removed from the root canal by the use of copious irrigation with either sodium hypochlorite (NaOCl) or saline combined with instrumentation and a final rinse with 17% ethylenediaminetetraacetic acid (EDTA). Yet very few studies have been performed to assess the efficiency of ultrasonic file irrigation on the removal of Ca(OH)₂ from root canal walls.[14-16]

Hence, the purpose of this study is comparative evaluation between hand instrumentation and ultrasonic file irrigation in removal efficacy of various Ca(OH)₂/chlorhexidine medicaments from the root canal with the help of Biological microscope and Digital Camera.

**Materials and Methods**

Sixty-four human single-rooted teeth with straight canals were used. Following extraction, the teeth were stored for 2 days at room temperature in 3% NaOCl to remove organic debris. Subsequently, they were scaled with ultrasonics, washed with distilled water for the removal of any calculus or soft tissue debris and then immersed in 10% formalin solution until use.

After access cavities were prepared a size 15 no. K-file (Dentsply Maillefer, Ballaigues Switzerland) was introduced into the canal until it was visible at the apical foramen. The working length was determined by subtracting 1 mm from this measurement. This same file was used during preparation, and it was introduced into the canal until it was visible at the apical foramen to ascertain patency at all times. The root canal preparation was performed using Pro-taper Universal for Hand use (Dentsply Maillefer, Ballaigues Switzerland) with shaping files SX, S1, and S2 and finishing files F1 and F2 with a crown-down technique. The instrumentation was standardized with an F2 reaching full working length and a final coronal flaring with size 2 and 3 Gates-Glidden drills (Mani, Japan). EDTA gel (Glyde Dentsply Maillefer, Ballaigues Switzerland) was used as a chelating agent and was introduced in the canal on the tip of each successive instrument. The canals were irrigated between instruments with 5 ml of 3% NaOCl (Astral Pharmaceuticals Pvt. Ltd. India). Irrigation was performed using 5 ml disposable plastic syringes with 27-gauge needle tips.

Teeth were divided randomly into experimental Groups A, B, and C of 21 teeth each, the remaining tooth remained untouched and served as a negative control. The root canals were dried with paper points and then filled with the experimental material.

- **Group A:** Teeth of Group A were filled with Ca(OH)₂ mixed with saline
- **Group B:** Teeth of Group B were filled with Ca(OH)₂ mixed with 0.2% chlorhexidine gel
- **Group C:** Teeth of Group C were filled with Ca(OH)₂ mixed with 0.12% chlorhexidine solution.

All medicaments used were mixed to a creamy consistency on a glass slab using Ca(OH)₂ powder and the respective solution or gel. Pastes were placed with a size 25 Lentulo paste carrier on a contra-angle handpiece until the medicament was visible at the apical foramen. The access cavities were temporarily sealed with a cotton pellet and a filling cavity (Prevest Denpro, India) to a depth of 3 mm. Teeth were stored at 37°C and 100% relative humidity for 7 days.

A tooth from each group served as a positive control and the three Groups (A, B, and C) were further divided into two subgroups (A₁, A₂), (B₁, B₂), and (C₁, C₂) of each group containing 10 teeth each.

In subgroups A₁, B₁, and C₁, the medicament was removed by hand instrumentation using the master apical file followed by irrigation with 5 ml of 3% NaOCl and 17% EDTA solution. In subgroups A₂, B₂, and C₂, medicament was removed with size 15 or 20 ultrasonic file mounted on a piezoelectric handpiece (Gulin woodpecker medical instrument Co., Ltd. China) at a power setting of three, was passively activated for 30s using 5 ml of 3% NaOCl and 17% EDTA solution as irrigants.

Using a cylindrical diamond bur in a high-speed handpiece, under constant water spray, all roots were longitudinally grooved on the buccal and lingual surfaces, preserving the inner shelf of dentine surrounding the canal, and split into two halves using a chisel and orthodontic plier.

**Evaluation**

Images of each half of the canal were acquired by a digital camera (Panasonic DMC-FZ18 Digital camera Matsushita Electric Industrial Co., Ltd. Japan) mounted on a biological microscope (Olympus, Japan) at 5X. A scoring system was defined to assess the quantity of residue on the canal walls. Evaluation scales used were as follows:

1. No visible remnants
2. Scattered remnants
3. Distinct masses
4. Densely packed remnants.

Remnants were evaluated in each third of all sections (Cervical, middle, and apical) and the highest score observed was recorded. Note the characteristic pattern of residual debris corresponding to score 2 at cervical third, 3 at middle third and score 4 at apical third after removal of Ca(OH)₂ paste in Group A by hand instrumentation [Figure 1]. Root canal after the removal of Ca(OH)₂/chlorhexidine gel (paste) in Group B by ultrasonic file irrigation [Figure 2].

**Statistical analysis**

Data were subjected to statistical interpretation using Mann–Whitney and Kruskal–Wallis test. Mann–Whitney
test showed a statistically significant difference between two groups $A/\text{A}_U$ at cervical ($P = 0.001$) and middle third ($P = 0.031$). In apical third, the difference was not statistically significant ($P = 0.218$) [Table 1]. While comparing the two groups $B/\text{B}_U$, the difference was statistically significant at cervical ($P = 0.000$), and middle third of root canal ($P = 0.000$) whereas in apical third the difference found was not statistically significant ($P = 0.866$) [Table 2]. Statistical analysis was done between the two groups $C/\text{C}_U$ at cervical, middle, and apical third. Statistically significant difference was found only in the middle third with a $P = 0.008$ [Table 3].

**RESULTS**

The results suggest that there was statistically significant difference ($P < 0.05$) found between ultrasonic file irrigation and hand instrumentation in removing various Ca(OH)$_2$/chlorhexidine containing medicaments at cervical and middle thirds of root canal and no significant difference ($P > 0.05$) at apical third of root canal.

**DISCUSSION**

In endodontics, the use of intracanal medicaments (Ca(OH)$_2$) is extremely important to promote disinfection of root canal walls where the eradication of microorganisms from canal irregularities is not possible by thorough biomechanical and chemical preparation. Although the exact mechanism of action of Ca(OH)$_2$ is still unknown, its antimicrobial activity is generally considered to be related to the release of hydroxyl ions in an aqueous environment producing a pH of approximately 12.5 even in dilute mixtures. The mechanism that Ca(OH)$_2$ uses to eliminate bacteria may include damage to the bacterial cytoplasmic membrane by inducing lipid peroxidation, protein denaturation, and damage to bacterial DNA and by serving as a physical barrier that withholds...
nutrients for bacterial growth and limits space for bacterial multiplication.\cite{18,19}

However, specific microorganisms have proved to be resistant to Ca(OH)$_2$. The mechanism involved in the resistance of E. faecalis to Ca(OH)$_2$ is as proton pumps are used by bacterial cells to maintain pH homeostasis within a narrow physiological range so that enzymes and proteins function normally. Evans et al.\cite{20} found that a functioning intracellular proton pump was the primary factor in alkaline resistance of E. faecalis, which showed a 20-fold to 70-fold reduction in survival at high pH. The proton pump of E. faecalis appears to function until it is overwhelmed at pH values of 11.5 and greater.

Ca(OH)$_2$ action is directly influenced by the release of hydroxyl ions and by inactivation of enzymes of the cytoplasmic membrane of microorganisms, which chemically alters the organic components and transport of nutrients causing toxic effects on the cells. Chemically, hydrosoluble vehicles (distilled water and saline) induce a higher speed of ionic dissociation than viscous (polyethylene glycol) and oily vehicles (camphorated paramonochlorophenol).\cite{21} Thus, vehicle of a hydrosoluble (saline) nature was selected for this particular study. Beltes et al. stated that to ensure continuous release of hydroxyl ions over time sufficient to achieve an antibacterial effect, a Ca(OH)$_2$ dressing has to be applied in the root canal for 7 days.

Chlorhexidine is a broad-spectrum antimicrobial agent that has been reported to be an effective medicament in endodontic therapy.\cite{22} As a root canal irrigant and intracanal medicament, chlorhexidine has an antibacterial efficacy comparable to that of NaOCl.\cite{23-26}

The effect of ultrasonic agitation of the irrigants has been evaluated with contradictory results.\cite{27,28} Two types of ultrasonic irrigation have been described in the literature: one where irrigation is combined with simultaneous ultrasonic instrumentation and another without simultaneous instrumentation, so-called passive ultrasonic irrigation. During ultrasonic instrumentation, the file is intentionally brought into contact with the root canal wall. Ultrasonic instrumentation has been shown to be less effective in removing simulated pulp tissue from the root canal system or smear layer from the root canal wall than passive ultrasonic instrumentation.\cite{29,30} Passive ultrasonic instrumentation relies on the transmission of acoustic energy from an oscillating file or smooth wire to an irrigant in the root canal. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming and cavitation of the irrigant. After the root canal has been shaped to the master apical file (irrespective of the preparation technique used), a small file or smooth wire (e.g. 15) is introduced in the center of the root canal, as far as the apical region. The root canal is then filled with an irrigant solution, and the ultrasonically oscillating file activates the irrigant. As the root canal has already been shaped, the file or wire can move freely, and the irrigant can penetrate more easily into the apical part of the root canal system, and the cleaning effect will be more powerful. A file larger than size 15 or 20 will only oscillate freely in a wide root canal. A size 25 file may in fact produce less acoustic streaming\cite{31,32} than a size 15 and 20 file.

In the present study, a size 15 or 20 file was used with the frequency of the oscillating instrument fixed at 30 kHz for removing various Ca(OH)$_2$/chlorhexidine medicaments from the root canal. In this study, an attempt was made to ensure a similar amount of the irrigant solution (5 ml of 3% NaOCl and 17% EDTA) during manual and ultrasonic irrigation techniques.\cite{33}

In the present study, a scoring system was used to facilitate comparison among groups instead of calculating the percentage ratio of medicament coated surface area to the total canal surface area as previously reported.\cite{34} Using a scoring system was considered a reliable method because of the difficulties in automatically selecting the areas covered with remnants with appropriate software, because of the color similarities between the chlorhexidine containing materials and some areas of dentine.

Concerning the control groups, one canal did not receive any Ca(OH)$_2$ containing medicament to ensure that analysis of clean canals did not yield false positives of remaining debris; likewise, three canals received Ca(OH)$_2$ containing medicament without subsequent removal to assure that Ca(OH)$_2$ containing medicament was uniformly present throughout the length of the canals and that the amount initially placed was significantly different from any amounts remaining after removal attempts.\cite{16}

The results of our study demonstrate a superior cleaning efficacy of ultrasonic file irrigation than hand instrumentation at cervical and middle third of the root canal. In the present study, it is possible that ultrasonic file was placed short of working length into the root canal whereas in the above study the irrigation

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**Table 3: Statistical analysis using Mann Whitney test between Group C/C$_0$ at Cervical, middle and apical third**

| Group C- Calcium hydroxide mixed with 0.12% Chlorhexidine solution | Mean Rank |
|---------------------------------------------------------------|-----------|
| Cervical Hand Instrumentation C$_i$ | 12.10 |
| Third Ultrasonic File Irrigation C$_U$ | 8.90 |
| Middle Hand Instrumentation C$_i$ | 13.60 |
| Third Ultrasonic File Irrigation C$_U$ | 7.40 |
| Apical Hand Instrumentation C$_i$ | 12.815 |
| Third Ultrasonic File Irrigation C$_U$ | 8.85 |

**Test Statistics**

| Test Statistics | Cervical Third | Middle Third | Apical Third |
|-----------------|----------------|--------------|--------------|
| Mann-Whitney U  | 34.000         | 19.000       | 33.500       |
| Wilcoxon W      | 89.000         | 74.000       | 88.500       |
| Z               | -1.446         | -2.668       | -1.407       |
| Asymp. Sig. (2-tailed) | 0.148 | 0.008 | 0.159 |
nal opening was introduced 1 mm short of working length and the needle opening was adjacent to the apical groove.

Furthermore, no statistical difference was found between the experimental groups at the apical third, probably because thorough cleaning of the most apical part of any preparation remains difficult, even with an ultrasonic device. Although an apical plug with Ca(OH)₂ has been advocated for its prolonged antimicrobial activity after filling of the canal space,[13] it is preferable to remove it as it might enhance apical leakage, as it is believed to be diluted in contact with tissue fluids.

The results of our study confirm those of previous studies by Lee et al., Vander Sluis et al., Balvedi et al., who found that ultrasonic file irrigation was more effective than hand instrumentation at cervical and middle third of the root canal. However, these results are based on measurements conducted over a short period using an in vitro study.

In view of the possible implications of the remnants of Ca(OH)₂ on the outcome of root canal treatment, more research has to be done on the mechanical or chemical means that will effectively remove it before final obturation of the root canal to reduce side effects and optimize the benefits of Ca(OH)₂ in endodontic treatment.

**Conclusion**

None of the techniques used in this study removed the interappointment root canal medicaments effectively. Remnants were found in all experimental groups regardless of the medicament used.

In short, the use of ultrasonic file irrigation was more effective than hand instrumentation for removing various medicaments from the root canal.

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**Conflicts of interest**

There are no conflicts of interest.

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