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Original Article

Evaluation of different irrigation solutions and activation methods on removing calcium hydroxide

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Abstract *Background/purpose:* Dual Rinse HEDP is a soft chelator which can be used simultaneously with sodium hypochlorite (NaOCl). The aim of this study is to evaluate the efficacy of Dual Rinse HEDP with different irrigation systems on removing calcium hydroxide [$Ca(OH)_2$].
Materials and methods: Eighty maxillary central incisor teeth were shaped and a standard groove on the apical third of the root canal surface was prepared. The root canals were filled with $Ca(OH)_2$. Samples were divided into two groups according to the solution and these two groups were divided into three subgroups based on the activation technique. In group 1 ($n = 10$) $Ca(OH)_2$ was removed using conventional irrigation with NaOCl whereas in group 2 ($n = 10$) conventional irrigation with NaOCl – Dual Rinse HEDP mixture was used. Group 3 ($n = 15$) and group 4 ($n = 15$) received sonic activation with the same irrigants as groups 1 and 2 respectively. In group 5 ($n = 15$) passive ultrasonic irrigation (PUI) was used with NaOCl while in group 6 ($n = 15$) the irrigant was the mixture. The amount of remaining $Ca(OH)_2$ in the artificial grooves were evaluated under a light microscope.
Results: None of the procedures managed to completely remove the $Ca(OH)_2$ from artificial grooves. There was no significant difference between the groups ($p = 0.053$). The scores were significantly lower in PUI group compared to the other techniques between NaOCl groups ($p = 0.021$).
Conclusion: Dual Rinse HEDP does not make a difference on elimination of $Ca(OH)_2$. PUI is more efficient than both methods when NaOCl solution is used.

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Introduction

Calcium hydroxide [$Ca(OH)_2$] is an intracanal medicament that has been benefited abundantly by dentists thanks to its high pH, antimicrobial activity, biocompatibility and capacity to provide hard tissue healing.[1,2] Remnant $Ca(OH)_2$ decreases the setting time of zinc oxide-based root canal cements, prevents infiltration of sealers into the dentinal tubules and indirectly causes apical leakage.[2,3] Hence total removal of $Ca(OH)_2$ is desired before the obturation of the teeth. However several studies have shown that complete removal of $Ca(OH)_2$ from the root canals does not exist with means of conventional treatment modalities.[4,5] There are different irrigation activation techniques to eliminate residual $Ca(OH)_2$ from the root canal system.

Several activation systems have been administered however none of them have provided complete elimination of $Ca(OH)_2$ from the root canal system. Passive ultrasonic irrigation (PUI) is one of the techniques used in order to obtain a more calcium hydroxide-free root canal system through acoustic streaming. Acoustic streaming produces shear stresses that can dissociate bacterial colonies and debris away from the canal walls.[6] It uses an ultrasonically activated file to transmit energy to the irrigation solution. Besides PUI, EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) (EA) is a device used with sonic activation of the irrigant. It is consisted of a portable handpiece and three sizes of tips that do not cut dentin.[7] It enables a safe fluid agitation thanks to its design.[8] Among several studies, a consensus has not been reached on which activation technique is more efficient.[7,9,10]

Apart from several activation systems, different irrigation solutions are used in order to achieve a more calcium hydroxide-free root canal system. Dual Rinse HEDP (Medcem GmbH, Weinfelden, Switzerland) is a mild chelating agent mostly used by mixing with sodium hypochlorite (NaOCl). It is in a capsule form and one capsule contains 0.9 gr of 1-hydroxyethane 1,1-diphosphonic acid (HEDP or HEBP or etidronic acid) powder as it is indicated by the manufacturer. When this powder in the capsule is mixed with NaOCl, free available chlorine and Na_4HEDP are formed which has proteolytic/antibacterial effects and sequesters calcium which prevents hard tissue accumulation respectively.[11,12] Smear layer can also be reduced using this agent.[13–15] Furthermore, studies have shown that this mixture can enhance the adhesion of some type of cements to the root canal dentin.[16–19] This combination can be used to remove $Ca(OH)_2$ dressing and get a clean root canal system as well considering the recommendation of the manufacturer. However, no study which aims to assess the efficacy of Dual Rinse HEDP on removing $Ca(OH)_2$ from the root canal system is present.

The objective of this study is to compare the capacity to remove $Ca(OH)_2$ from standardized artificial grooves in straight root canals of NaOCl and Dual Rinse HEDP using ultrasonic and sonic activation. The null hypothesis was that there will be no difference in the efficacy of NaOCl and its combination with Dual Rinse HEDP on the removal of $Ca(OH)_2$ intracanal medicament.

Materials and methods

Ethical approval was obtained from the Clinical Research Ethics Committee of the Faculty of Dentistry of Marmara University (project number: 2018-213).

Sample selection

Eighty single-rooted permanent human maxillary central incisors extracted for periodontal reasons were selected according to the inclusion criteria. Inclusion criteria involved single and straight canals, intact apices and roots longer than 15 mm. All teeth were radiographed to confirm that teeth had neither resorption, previous root canal treatment nor root canal calcification. After the extraction, teeth were kept in 2% thymol solution at room temperature.

Specimen preparation

The access cavities were prepared using diamond round burs. #10 K-file (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) was inserted into the canal until its tip was visible at the apical foramen. Working length was decided as 1 mm short from the measurement of the initial file. The root canals were shaped using ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) files up to size ×3. Within the instrumentation procedure, the canals were irrigated with 2 ml of 1% NaOCl (Endosolve-HP, Imicryl, Konya, Turkey) via a dental syringe (Beybi, Istanbul, Turkey) with 30-gauge slotted-end needle (CanalPro Flex Tips, Coltene, Langenau, Germany) between each file. Following the finalization of the mechanical preparation, the canals were rinsed with 5 ml of 17% EDTA (Promida, Eskişehir, Turkey) and 5 ml of 1% NaOCl both for a minute separately. Finally, they were flushed with 5 ml of distilled water with a 30-gauge needle and dried with sterile paper points.

The teeth were placed in Eppendorf vials (Tıp Kim San, Istanbul, Turkey) with silicon impression material (Zetaplus, Zhermack, Badia Polesine, Italy). Following the setting, the teeth were removed, and longitudinal grooves were prepared on the labial and palatinal surfaces of the teeth. The teeth were split in half with the help of a chisel. On one of the root halves, a standard longitudinal groove of 3 mm in length was created at 3–6 mm from the apex, using a hand spreader (A60, Dentsply Maillefer, Ballaigues, Switzerland) with its working portion removed and its shank sharpened to V shape, based on the model described by Lee et al.[20] These 0.5 mm deep and 0.2 mm wide grooves were intended to represent the uninstrumented canal irregularities in the apical third. The grooves were filled with a water-based calcium hydroxide paste (Procal R, Promida, Eskişehir, Turkey). The roots were reassembled and placed in their own Eppendorf vials. Following placement, the root canals were completely filled with calcium hydroxide paste with the help of NaviTip delivery tips (Ultradent, South Jordan, UT, USA). The paste was injected into the middle third and carried deeper by moving these tips in apical—coronal directions. This procedure was repeated until coronal third was filled with $Ca(OH)_2$. The access cavities were temporarily sealed using wet cotton pellet and cavit (Nucavfil, PSP Dental, Kent, United Kingdom). The teeth were radiographed to ensure complete filling of the roots. The Eppendorf vials containing the teeth were stored for a week in 37 °C, 100% humidity. After 1-week, temporary fillings and cotton pellet were removed with a diamond round bur and a dental tweezer respectively. The calcium hydroxide in the root canal was loosened and enough space for irrigation tips was prepared with a #15 K-file (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). Specimens were divided into two groups according to the irrigant used for elimination of calcium hydroxide. These two groups were divided into three subgroups according to the irrigation activation methodology.

Manual activation (MA)

In group 1 each root canal was irrigated with 5 ml in total of 1% NaOCl, meanwhile in group 2 each root canal was irrigated with 5 ml in total of 1% NaOCl and Dual Rinse HEDP mixture. Both groups received manual activation and for this purpose a 30-gauge slotted-end needle was moved in apical—coronal directions.

Sonic activation

In group 3 each root canal was irrigated with 5 ml in total of 1% NaOCl, where in group 4 each root canal was irrigated with 5 ml in total of 1% NaOCl and Dual Rinse HEDP mixture. The irrigant solution in both groups was sonically activated with EndoActivator System. EndoActivator System was used with a red (#25/0.04) tip set at 10,000 cycles/min.

Ultrasonic activation

In group 5 each root canal was irrigated with 5 ml in total of 1% NaOCl, in the meantime each root canal of group 6 was irrigated with 5 ml in total of 1% NaOCl and Dual Rinse HEDP mixture. Both groups received passive ultrasonic irrigation with VDW Ultra (VDW GmbH, Münich, Germany). Irri S 21/25 file was used, and the power of the device was set to 30.

In all groups the irrigation tips were placed within 2 mm short from the working length. The activation in each of the groups was done three times for 20 s. Each time the solution was renewed. Dual Rinse HEDP — NaOCl mixtures were prepared freshly by adding a capsule of Dual Rinse HEDP into 10 ml of 1% NaOCl solution and stirring the solution for a minute. As the mixture is not storable for a long time[15,21]; immediately after the preparation, each 10 ml mixture were divided equally into two syringes and used for two specimens. After the removal of calcium hydroxide procedures, all canals were rinsed with 5 ml of distilled water with a 30-gauge needle and dried with sterile paper points.

The teeth were removed from the Eppendorf vials, split in half again and observed under a light microscope (ZEISS Axio Imager 2, Carl Zeiss Microscopy GmbH, Göttingen, Germany) at 50× magnification. The images were captured with a digital microscope camera (Axiocam ICc 5, Carl Zeiss Microscopy GmbH, Jena, Germany) attached to the light microscope. As a result of the working principle of light microscopy and the 3-dimensional structure of the specimens, 4 separate images each with different parts focused on with same framing (without changing the camera or specimen position) were captured. These images were superimposed (Adobe Photoshop CC Software, Version: 2017 Adobe systems, San Jose, CA, USA) and final images were formed (Fig. 1). The grooves were scored by three dentists according to a scoring system:

Score 0: The groove is empty; Score 1: Less than half of the groove is filled with $Ca(OH)_2$; Score 2: More than half of the groove is filled with $Ca(OH)_2$; Score 3: The groove is filled completely with $Ca(OH)_2$.[22]

Statistical analysis

Kendall W was used to assess the agreement between three raters. The p-value did not exceed 0.022 in any group showing a consistency in scoring. Therefore, the scores that have been agreed upon by at least two out of three raters

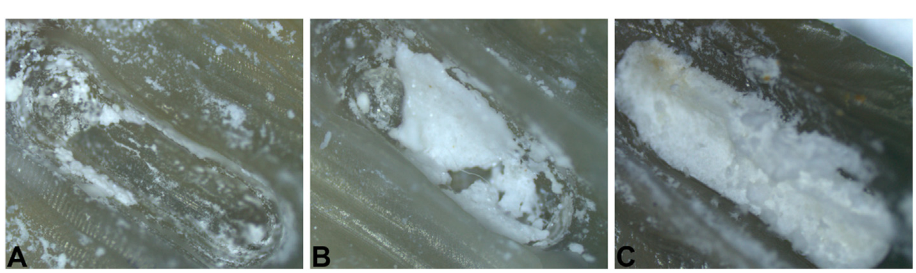

Figure 1 Representative light microscope images of the artificial grooves after the superimposition procedure (magnification 50×). (A) Score 1, (B) score 2, (C) score 3.

were used at statistical analysis. The chi-square test was performed for the analysis of the removal of $Ca(OH)_2$ as a result of the ordinal scoring system. The level of significance was set at 0.05. Statistical analyses were performed with IBM SPSS 20 software (SPSS Inc., Chicago, IL, USA).

Results

Chi-square test showed that scores were not affected by the irrigation solution and activation technique statistically ($p = 0.053$). Regarding the groups that used NaOCl as the solution; ultrasonic activation removed significantly more $Ca(OH)_2$ from the grooves than sonic and manual groups ($p = 0.021$). Whereas in the Dual Rinse HEDP groups none of the activation methods outperformed another ($p = 0.198$) (Table 1). In the groups that used sonic and ultrasonic activation, the solution type did not make any significant difference ($p = 0.574$ and $p = 0.079$ respectively). However despite the fact that a statistical difference could have not been reached ($p = 0.061$), the mixture removed more $Ca(OH)_2$ from the grooves than NaOCl solution when the manual activation was applied (Table 2).

Discussion

Calcium hydroxide is still one of the most used materials in operative dentistry despite the newly developed materials and products. However residual $Ca(OH)_2$ prior to obturation is undesirable as it disrupts the setting and sealing capacity of zinc-based sealers and indirectly causes apical leakage.[2,3] It has not been concluded that which irrigation solution and activation method gives the best result for a calcium hydroxide-free root canal system. In order to find an answer to this question numerous studies have been done using different imaging, scoring, irrigation activating methods and irrigation solution on various study models.[23–26] Yet the current study is the first evaluating the removal of $Ca(OH)_2$ with ultrasonic and sonic activation of Dual Rinse HEDP using artificial groove model and a light microscope.

The use of different kinds of visualization devices such as SEM, stereomicroscope, light microscope, digital camera and CBCT diversified the ways to reach the ideal irrigation system for removal of intracanal medicaments.[25–27] The light microscope used in this research and the digital microscope camera attached to it provided the images. Superimposing four separate images of the exact same area with different parts focused on provided a more 3-dimensional view which enabled the raters to clearly see the borders of the artificial grooves and score the volume of remaining $Ca(OH)_2$.

The study design described by Lee et al. has been used in several researches as a result of its high intra-observer reproducibility and the ability to standardize the size and location of the groove and volume of $Ca(OH)_2$.[4,20,28] Despite these advantages, this model fails to represent the complexity of root canal anatomy and address the medicament diffused into the dentinal tubules.[29] Furthermore, the process of dividing teeth into two pieces may prevent formation of a perfectly restricted root canal as there may be micro holes between two parts although they were brought together in a silicon impression.[29] This may decrease the potential pressure formed in the root canal and reduce the effect of the irrigation system but the fact that artificial grooves are easier to be cleaned than isthmuses and canal irregularities in vivo,[28] may cancel out the reduced pressure effect.

Recent literature shows that Dual Rinse HEDP resulted significantly higher surface tension and push-out bond strength than EDTA.[30,31] While studies evaluating the product's active ingredient "HEDP" proved it has some

Table 1 Residual $Ca(OH)_2$ scores according to the irrigation solution used.

	Score	0	1	2	3	p
Irrigant	Activation	n (%)	n (%)	n (%)	n (%)	
NaOCl	Manual	0 (0)	0 (0)	3 (30)	7 (70)	0.021*
	Sonic	0 (0)	0 (0)	5 (33.3)	10 (66.7)	
	Ultrasonic	0 (0)	5 (33.3)	6 (40)	4 (26.7)	
NaOCl + Dual Rinse HEDP	Manual	0 (0)	4 (40)	3 (30)	3 (30)	0.198
	Sonic	0 (0)	1 (6.7)	4 (26.7)	10 (66.7)	
	Ultrasonic	0 (0)	3 (20)	2 (13.3)	10 (66.7)	

* Chi-square ($p < 0.05$).

Table 2 Residual $Ca(OH)_2$ scores according to the activation method used.

	Score	0	1	2	3	p
Activation	Irrigant	n (%)	n (%)	n (%)	n (%)	
Manual	NaOCl	0 (0)	0 (0)	3 (30)	7 (70)	0.061
	NaOCl + Dual Rinse HEDP	0 (0)	4 (40)	3 (30)	3 (30)	
Sonic	NaOCl	0 (0)	0 (0)	5 (33.3)	10 (66.7)	0.574
	NaOCl + Dual Rinse HEDP	0 (0)	1 (6.7)	4 (26.7)	10 (66.7)	
Ultrasonic	NaOCl	0 (0)	5 (33.3)	6 (40)	4 (26.7)	0.079
	NaOCl + Dual Rinse HEDP	0 (0)	3 (20)	2 (13.3)	10 (66.7)	

strengths over EDTA such as on antimicrobial activity, effect on bonding, and stem cell migration and relationship with NaOCl.[16,18,32–34] Taking these outcomes into consideration, the capsule form of etidronate may be both more convenient as it is applied with NaOCl in a single mixture and more effective compared to usual chelation agents such as EDTA. Thus, regarding these results, Dual Rinse HEDP may be considered as a potential successor of EDTA after various trials. Despite these facts, studies that include the active ingredient (HEDP/etidronate) — not the commercial product — as a $Ca(OH)_2$ removal solution are few.[23,35,36] This study varies as it assesses removal of $Ca(OH)_2$ with the capsule form of HEDP for the first time in the literature.

Results of this study indicated that Dual Rinse HEDP did not show a statistically significant difference compared to NaOCl in any of the activation techniques. This result contradicts the results of the current literature, as HEDP outperformed NaOCl on removing $Ca(OH)_2$ in three studies.[23,35,36] However, in the present study when syringe irrigation was used, the scores of the chelation group were noticeably lower than the NaOCl group. The $Ca(OH)_2$ in the grooves remained untouched in 70% of the teeth in NaOCl group, but only 30% were completely filled in the capsule group; meaning it performed better than NaOCl. The reason theorized why it did not reach to a statistical significance ($p = 0.061$) was that the number of samples in first and second group was below an ideal limit ($n = 10$). Besides, the scoring system of van der Sluis et al. was used in this study as it had been a reference in these kind of researches for many years yet it was thought it would be applicable to this study as well. Though it would possibly make a statistical difference in favor of the capsule if quantitative scales would have been used as they enable more accurate separation of samples. Additionally, the three studies mentioned above, all included the solution version with different pH of the active gradient, not the capsule form as in this study. Therefore, using HEDP based products might be a better choice when aiming a medicament-free root canal system if an activation device is not present.

PUI is one of the most effective methods when a relatively cleaner root canal system is desired. This present study showed that if NaOCl is the choice as the irrigation solution, ultrasonic activation helps clinicians to achieve a clean root canal system more than sonic or manual activation. Current literature supports this argument with quantities of research.[7,37,38] On the other hand, in the Dual Rinse HEDP group the scores were distinguishably high compared to NaOCl group with PUI despite the fact that there was no significant difference between them. The expectation was that PUI and the capsule would give the best combination however contrarily this group displayed 66.67% of complete presence of $Ca(OH)_2$, while NaOCl group had only 26.67% of score threes. Within the limitations of this study, this could be explained by ultrasonic energy's ability to generate heat in the solution, which leads the salt in the mixture to reprecipitate. This reprecipitation may eliminate the advantage of cleaning ability of the ultrasonic activation resulting this performance. This explanation needs to be evaluated by farther researches. In addition, two EndoActivator groups almost received the exact same scores displaying that both solutions performed similarly.

Both sonic and manual activation did not differ statistically on ability of removing $Ca(OH)_2$ regardless the solution used in this research. This is in agreement with different studies.[7,37,39]

All in all, it is clear that none of the irrigation solution and activation technique have been able to completely remove $Ca(OH)_2$ both in this study and other researches that have dealt with this concept. Nevertheless, PUI should be the method of choice if NaOCl is used as the irrigant while removing the intracanal medicament. Despite the fact that Dual Rinse HEDP's effect could not reach to a statistical significance, the scores in the conventional irrigation group are promising and the results show that more detailed studies conducting on higher number of samples, using more diverse irrigation systems with quantitative evaluation scales are needed to analyze this product comprehensively.

Authorship statement

Conception and design of study: Z.H. Cimilli, S. Harzivartyan, A.B. Hazar, N. Kartal.

Acquisition of data: Z.H. Cimilli, S. Harzivartyan, A.B. Hazar.

Analysis and/or interpretation of data: Z.H. Cimilli, S. Harzivartyan, N. Kartal.

Drafting the manuscript: Z.H. Cimilli, S. Harzivartyan.

Revising the manuscript critically for important intellectual content: Z.H. Cimilli, N. Kartal, A.B. Hazar.

Approval of the version of the manuscript to be published: Z.H. Cimilli, S. Harzivartyan, A.B. Hazar, N. Kartal.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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