

REMOVAL OF DOUBLE ANTIBIOTIC PASTE AND CALCIUM HYDROXIDE FROM SIMULATED MODELS OF REGENERATIVE ENDODONTIC PROCEDURES USING SEVERAL PROTOCOLS OF IRRIGATION: *IN-VITRO* COMPARISON STUDY

Mohammad Yaman Seirawan¹, Kinda Ilayous¹, Mohammad Kinan Seirawan^{2,3}, Mazen Doumani^{1,4}

¹Department of Restorative and Endodontics, Faculty of Dentistry, University of Damascus, Damascus, Syria

²Department of Removable Prosthodontics, Faculty of Dentistry, University of Damascus, Damascus, Syria

³Department of Prosthetic Dental Sciences, Al-Farabi College for Dentistry and Nursing, Riyadh, Saudi Arabia

⁴Department of Restorative Dental Sciences, Al-Farabi College for Dentistry and Nursing, Riyadh, Saudi Arabia

ABSTRACT

INTRODUCTION: Although medicinal dressings are essential in regenerative endodontic procedures, they may adversely affect stem cells viability of periapical papilla.

OBJECTIVES: This study was conducted to compare the efficacy of different irrigation protocols on dressings removal from root canal undergoing regenerative endodontic procedures (REPs).

MATERIAL AND METHODS: A total of 69 single canal teeth were shaped and standardized in length. Irrigation was performed according to the 2018 recommendations of the American Association of Endodontics and then, the apical part was enclosed by resin-modified glass ionomer (RMGI). Double antimicrobial paste (DAP) was applied at a concentration of 1 mg/ml in group 1 ($n = 30$), followed by coronal sealing by RMGI, whereas calcium hydroxide $\text{Ca}(\text{OH})_2$ was used in group 2. Group 3 ($n = 6$) was fully filled by both of dressings as a positive group, while group 4 ($n = 3$) was empty as a negative group. The samples were preserved in humidity for 3 weeks; group 1 and group 2 were divided into 3 sub-groups ($n = 10$) (EDTA and ultrasonic, EDTA and sonic, and EDTA and hybrid activation; ultrasonic followed by sonic activation), and the irrigation was performed. Teeth were split longitudinally, and the residues were evaluated under 40x microscopic magnification. Data were collected, and the results were recorded and statistically analyzed using Kruskal-Wallis test at a confidence level of 95%.

RESULTS: Hybrid activation was the most effective protocol for removal of both dressings in the apical third, followed by sonic irrigation with no statistical differences, and by passive ultrasonic irrigation with statistical differences in DAP group.

CONCLUSIONS: Hybrid activation showed predictable removal of residues, but there was no protocol that could completely remove the dressing from the canal.

KEY WORDS: ultrasonic, sonic activation, double antibiotic paste, regenerative endodontic procedures.

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ADDRESS FOR CORRESPONDENCE: Dr. Mohammad Yaman Seirawan,
Department of Restorative and Endodontics, Faculty of Dentistry, University
of Damascus, Damascus, Syria, e-mail: yamansr@hotmail.com

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INTRODUCTION

Endodontic regenerative procedures depend on revascularization and renewal of dentin-pulp complex in immature teeth injured by dental caries or trauma, leading to a partial or complete pulp death. Pulp regeneration requires several conditions, in which, the most important is to ensure maximum sterility of root canal and ideal coronal sealing to prevent bacterial leakage and return of infection. Sterile environment using chemical irrigation and provisional medical dressing are necessary for the pulp regeneration [1]. One of the most renowned medicaments used in this field is calcium hydroxide dressing and triple antimicrobial paste (TAP) containing minocycline, which causes discoloration. Therefore, in order to overcome this problem, TAP was replaced by double antimicrobial paste (DAP) [2]. However, despite the medical benefit of DAP dressing, it may adversely affect stem cells viability of periapical papilla, if used at high concentrations or prolonged periods [3].

Several studies were conducted to assess the possibility of removing medical dressing from root canals, but the complexity of structure of the root canal system makes it difficult for irrigants to remove such dressing, particularly due to a potential penetration through dentinal tubules and their accumulation in lateral canals, anastomosis, and irregular areas in the root canal. These had led to the use of several methods to improve the ability of solutions in dressing removal, and the most common techniques are ultrasonic and sonic activations, which demonstrated the ability of sodium hypochlorite to remove medical dressing [4, 5]. Even though the recommendations for medical dressing removal from the canals were based on a copious irrigation of sodium hypochlorite and EDTA [6], the recent recommendations of the American Association of Endodontists propose using EDTA alone (without sodium hypochlorite) to reduce the cytotoxicity of sodium hypochlorite to stem cells of apical papilla [7]. Ultrasonic devices had been introduced to canal debridement in 1957, with high energy frequencies (25-30 kHz) and low tip movement amplitudes. Later on, sonic devices came up with lower frequencies (1-3 kHz) and minimum shear stresses, but the tip movement amplitude was clearly visible and wide [8].

The activation of irrigants, especially with ultrasonic systems, presents better canal debridement activity over using needle of irrigation, and better removal of calcium hydroxide comparing with syringe irrigation [9-12].

Each method of activation has its pros and cons, which may limit their use, where the ultrasonic tip touches the walls of a canal during nearly 20% of the entire time of procedure, and that could constrain the tip oscillation leading to a decrease of capacity of agitation. Probably, the same thing occurs during a sonic activation, which could impede the effective cleaning within the apical third. Another limitation of ultrasonic activation in curved canals, where oscillating can be lim-

ited, are the steel ultrasonic tips, which are harder than dentin and might distort the walls of canals. Therefore, the recommendations for their use can be restricted for a final irrigation or among straight canals, whereas sonic tips are made of flexible polymer components that never deform the texture of canal, as they are safely used within curved canals [8].

The current *in-vitro* study was comparing the effectiveness of three different protocols of irrigation activation with liquid EDTA in removing DAP and ready-made calcium hydroxide $\text{Ca}(\text{OH})_2$ dressing from root canals intended for regenerative endodontic procedures.

MATERIAL AND METHODS

The present study was conducted in dental laboratories of the Damascus University, Faculty of Dentistry, from 8th September 2019 to 7th November 2019. Ethical approval for the study method and protocol was obtained from institutional ethical review committee of the Damascus University prior starting the study.

A total of 69 roots with single canal, in a similar size and length, were selected from a collection of upper incisors and lower premolars. Teeth were cleaned out of soft tissue and calculus using ultrasonic scaler. Access cavity preparation was done, and patency was established. Working length with glide path was confirmed by K-file 15, cleaning and shaping were obtained by rotary system (ProTaper Universal; Dentsply Maillefer, Switzerland), and all canals were prepared up to F3 size to allow sufficient irrigation to all thirds of canals. Irrigation was performed with 1.5% sodium hypochlorite between each file using 30-gauge side-vented needle. According to the recommendations of the American Association of Endodontic, a solution of 1.5% sodium hypochlorite followed by a normal 0.9% saline were used and then, 17% ethylenediaminetetraacetic EDTA solution was applied to ensure the removal of smear layer. The final irrigation was executed with saline [7].

The apical tip and crown of teeth were sectioned under water coolant to unify the length of samples at 16 mm. After that, the canals were irrigated by saline and dried with paper points. The last 2 mm in the apical third was prepared by a diamond bur, and cylindrical cavity was sealed by resin-modified glass ionomer RMGI (Ionoseal; VOCO GmbH, Germany). Subsequently, the roots were randomly divided into four groups (Figure 1).

GROUPING

The samples were divided into 4 groups; group 1 ($n = 30$ roots) was filled with double antimicrobial paste DAP (metronidazole and ciprofloxacin), 1 mg/ml concentration, group 2 ($n = 30$ roots) was filled with ready-made calcium hydroxide $\text{Ca}(\text{OH})_2$ (Metapaste, Meta-biomed, Korea). In group 3 ($n = 6$ roots), 3 roots were

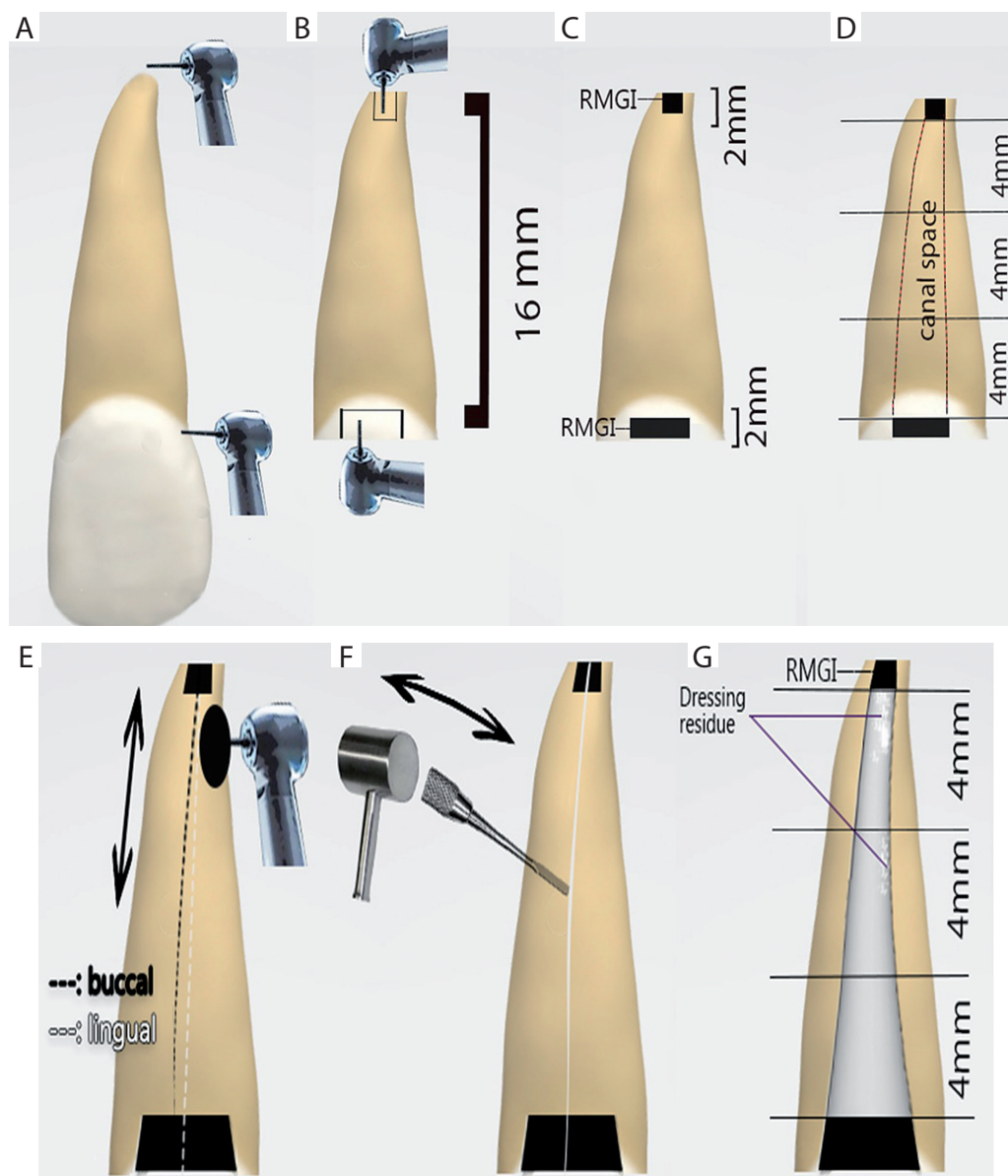


FIGURE 1. Preparing of the roots and samples. **A)** Apical and coronal refinement. **B)** Cavities drilling. **C)** Apical and coronal filling. **D)** Virtual root partitioning. **E)** Buccal and lingual grooving. **F)** Splitting by chisel and mallet by moderate forces. **G)** Virtual evaluation of residues

filled with DAP, 3 roots with Ca(OH)_2 with no intention of removing the dressing, and the remaining 3 roots were not filled and grouped in group 4.

The coronal cavities of all samples were sealed using RMGI, and the samples were preserved in humid environment for 3 weeks; then, the groups 1 and 2 were reopened from the coronal site.

DRESSINGS REMOVAL BY IRRIGATION WITH ULTRASONIC ACTIVATION: PROTOCOL 1

Ten roots from the groups 1 and 2 were washed out with 20 ml 17% EDTA solution using side-vented irrigation needles (Steri irrigation tips; Diadent, Korea) in

continuous up and down motions, with ultrasonic activation after every 5 ml of irrigation for 30 seconds, according to previously suggested clinical protocol [12]. Here, 0.25 mm tips were used (IrriSafe; Satalec Acteon, France) attached to an ultrasonic handpiece (Suprasson P5 Booster, Satalec Acteon, France), 1 mm of the working length and without touching the walls to enable free vibration. Then, drying with paper points was done.

DRESSINGS REMOVAL BY IRRIGATION WITH SONIC ACTIVATION: PROTOCOL 2

Ten roots from the groups 1 and 2 were washed out by the same above-mentioned irrigation with sonic acti-

vation after every 5 ml of irrigation for 30 seconds, using 0.25 mm tips (EQ-S; Metabiomed, Korea), 1 mm of the working length.

DRESSINGS REMOVAL BY IRRIGATION WITH HYBRID ACTIVATION: PROTOCOL 3

Ten roots from the groups 1 and 2 were washed out by the same above-mentioned irrigation with ultrasonic activation after every 5 ml of irrigation for 15 seconds, followed by sonic activation for 15 seconds with the same other details.

SPLITTING THE SAMPLES

The samples were split up longitudinally using a chisel and mallet after profound grooves were made on the buccal and palatal surfaces of the roots without canal invasion, using sharp incisive discs, according to previously suggested clinical protocol [5]. The finer half of each sample was selected for inspection under 40x microscopic magnification (Olympus CX21FS2, Japan) (Figure 1).

DRESSINGS RESIDUE EVALUATION

The longitudinal sections of the roots were compared on three levels (coronal, middle, and apical thirds) in terms of the presence of dressing residue by three independent examiners, and the degree of dressing remnant in the majority agreement at each stage was admitted.

The scoring criteria for residues evaluation were adopted as follows [13]:

- score 0: the canal is semi empty; less than 25% of the canal is covered with dressing residue (high cleanliness);
- score 1: less than half (25-50%) of the canal is covered with residue (partial cleanliness);
- score 2: more than half (50-75%) of the canal is covered with residue (slight cleanliness);
- score 3: the canal is covered completely (75-100%) with residue (no cleanliness).

Canal cleanliness was verified under stereomicroscope at $\times 40$ magnification; the numbered sections are shown in Figure 2 according to a previous scale.

STATISTICAL ANALYSIS

SPSS software was used to evaluate data (PASW Statistics 13; SPSS, Inc., Chicago, IL, USA), and the data were analyzed by means of Mann-Whitney test to evaluate the residues in three thirds of the canal (coronal, middle, apical) after irrigation using the three methods. The level of statistically significant difference was set at the confidence level of 95% and p -value = 0.05. The difference in removal ability between two types of dressing, DAP – Ca(OH)₂, was also studied.

RESULTS

There were significant differences between the two control groups and the two experimental groups. The re-

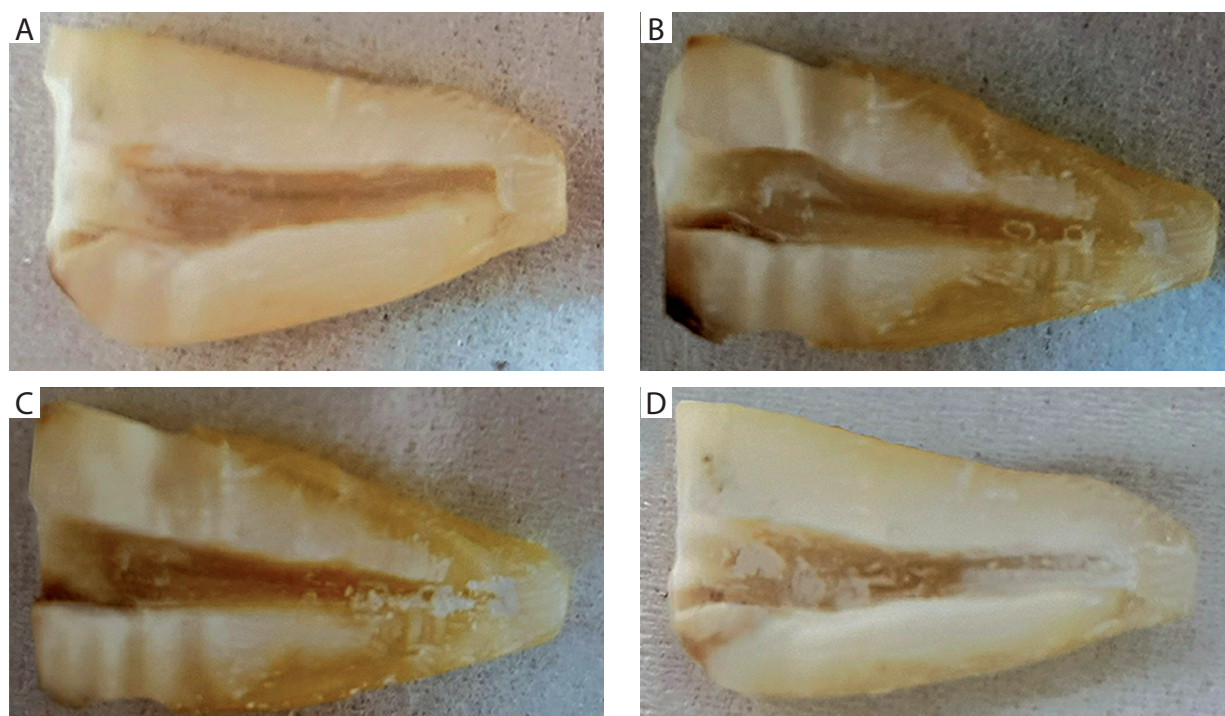


FIGURE 2. The longitudinal sections with degrees according to scale

TABLE 1. Mann-Whitney test results of three irrigation protocols

Dressing type/Third/ Irrigation protocol	Test value	p-value	Decision
DAP			
Coronal third			
Ultrasonic	-1.000	0.317	No significant difference
Sonic			
Ultrasonic	-1.000	0.317	No significant difference
Hybrid			
Sonic	0	1.000	No significant difference
Hybrid			
Middle third			
Ultrasonic	-1.076	0.282	No significant difference
Sonic			
Ultrasonic	-1.834	0.067	No significant difference
Hybrid			
Sonic	-0.890	0.374	No significant difference
Hybrid			
Apical third			
Ultrasonic	-1.076	0.282	No significant difference
Sonic			
Ultrasonic	-2.554	0.011	Significant difference
Hybrid			
Sonic	-1.646	0.100	No significant difference
Hybrid			

sults in Table 1 show that there were no statistically significant differences between ultrasonic activation and sonic activation in removal of both dressings (DAP – Ca(OH)₂) from each third of the canals (coronal, middle, apical), with *p*-value of 0.282-1 > 0.05.

The results in Table 1 demonstrate that there were no statistically significant differences between ultrasonic activation and hybrid activation in the removal of DAP residue from coronal and middle thirds of the canals (*p*-value = 0.067-0.317 > 0.05), but there were statistically significant differences in the apical third (*p*-value = 0.011 < 0.05) (Figure 3).

There were no statistically significant differences between ultrasonic activation and hybrid activation in removal of the residue of Ca(OH)₂ from three thirds of the canals, *p*-value = 0.240-1 > 0.05.

The results in Table 1 show that there were no statistically significant differences between sonic activation and hybrid activation in removal of both dressings from three thirds of the canals, *p*-value = 0.100-1 > 0.05 (Figure 4).

The results in Table 2 demonstrate that there were no statistically significant differences between removal ability of two types of dressings (DAP – Ca(OH)₂) among

TABLE 1. Cont.

Dressing type/Third/ Irrigation protocol	Test value	<i>p</i> -value	Decision
Ca(OH) ₂			
Coronal third			
Ultrasonic	0	1.000	No significant difference
Sonic			
Ultrasonic	0	1.000	No significant difference
Hybrid			
Sonic	0	1.000	No significant difference
Hybrid			
Middle third			
Ultrasonic	−0.457	0.648	No significant difference
Sonic			
Ultrasonic	−0.503	0.615	No significant difference
Hybrid			
Sonic	−0.951	0.342	No significant difference
Hybrid			
Apical third			
Ultrasonic	−0.247	0.805	No significant difference
Sonic			
Ultrasonic	−1.174	0.240	No significant difference
Hybrid			
Sonic	−1.023	0.306	No significant difference
Hybrid			

three protocols of irrigation (ultrasonic, sonic, hybrid) from three thirds of the canals (coronal, middle, apical), *p*-value = 0.067-1 > 0.05 (Figure 5).

DISCUSSION

The remaining medicinal dressing on the walls of root canal can adversely affect the success of regenerative endodontic treatment, so the dressing must be removed as much as possible before bleeding or application of scaffolds in the root canals [14].

The efficacy of dressing removal using irrigants relates to their ability to dissolve organic and inorganic tissues [9]. Since the canals in the current study were previously exposed to sodium hypochlorite, the organic tissues were no longer an obstacle, whereas the inorganic tissues integrated with dressing were the main obstacle. There were no statistically significant differences between NaOCl and EDTA in debris removal from artificial root canals regarding mechanical movement of the liquid, which was considered more important than its chemical action [8], while EDTA was significantly more effective

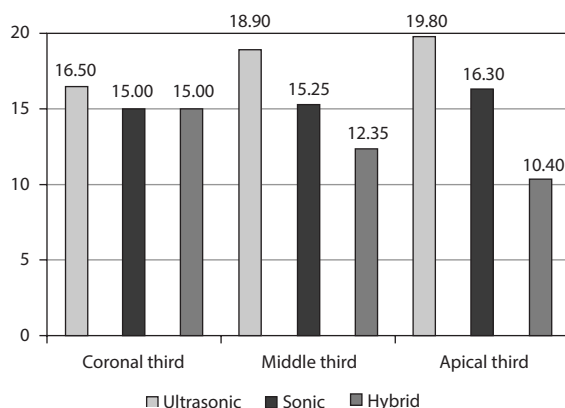


FIGURE 3. Differences of ranks means in pairwise comparisons of double antimicrobial paste residue

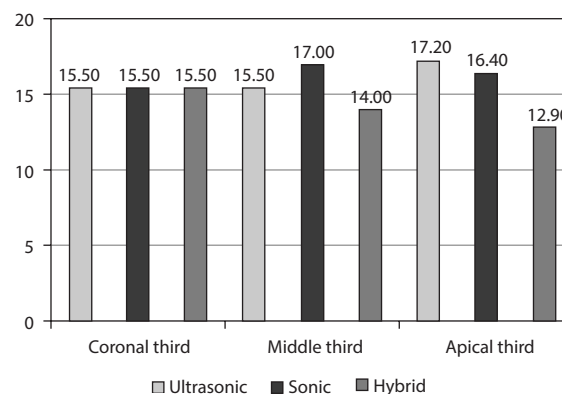


FIGURE 4. Differences of ranks means in pairwise comparisons of Ca(OH)_2 residue

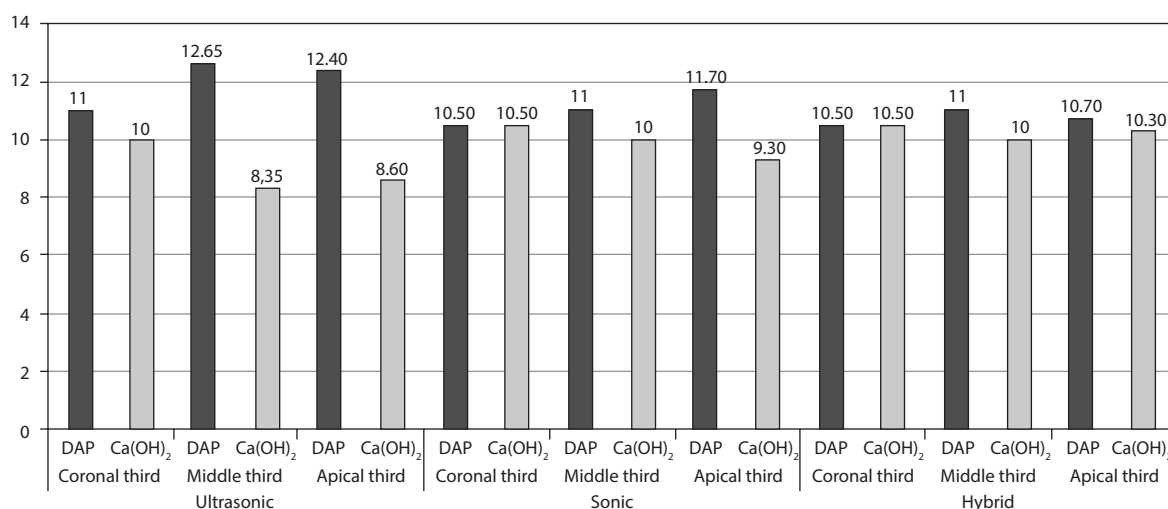


FIGURE 5. Differences of ranks means in pairwise comparisons of removal ability of the two dressings residue

than NaOCl in removal of Ca(OH)_2 dressing [15]. All that make 17% EDTA almost ideal solution for washing and removing the dressing due to its superior ability to chelate the inorganic structure.

In 2014, Berkhoff *et al.* [16] observed that it was difficult to remove triple antimicrobial paste from root canals due to its ability to spread and high stability within the root canal; this occurs in double antimicrobial paste, as there was no difference between removal of TAP and DAP according to recent studies [4, 5]. The dressing of calcium hydroxide was easier to be removed than TAP, especially with manual suspension mixed with saline or aqueous liquids, while the premixed pastes with oils or viscous substances, such as polypropylene glycol, could be removed less effective than aqueous suspensions [17]. This is consistent with the current findings in terms of the convergence of ability to remove DAP and Ca(OH)_2 , since the premixed type of Ca(OH)_2 with polypropylene glycol was used in the current study.

Activation in general increases the velocity of irrigants by transferring the energy generated by vibrations

of the oscillating tip to the irrigants inside the canal. That increases the irrigants ability to reach most anatomical problems [10], and regenerates the irrigants within the canal. In particular, passive ultrasonic irrigation (PUI) creates effects of acoustic streaming and cavitation for the irrigant, which provides better cleaning of canals than passive sonic irrigation (SI) [18].

Even though the power of ultrasonic oscillation ranges between 20 and 40 kHz, which exceeds the sonic power limited to 0.166-3 kHz, preponderance of one of them remains a controversial issue [8]. Other sonic systems were introduced with a high frequency of 6 KHz, driven by an air handpiece, resulting in similar ultrasonic effects as “cavitation and acoustic streaming”, which could enhance their capacity [19].

Recently, a sonic activation device EQ-S (Meta-biomed, Korea) was introduced, equipped with much more flexible, super elastic tips comparing to other systems. It is distinguished by multi-directional movement compared to linear direction movement in other systems, in addition to high speed in the built-in motor,

reaching 13,000 RPM, which may enhance the capacity of debridement and liquids activation in a 3D effective movement [20].

Most of the researches showed favorable results for the ultrasonic activation compared to sonic activation [12, 21]; for example, the ultrasonically irrigated canals with IrriSafe tips were cleaner than the sonically irrigated canals with EndoActivator, because PUI vibration frequency (30 KHz) was greater than SI frequency [18].

Ultrasonic activation improves the ability of sodium hypochlorite to remove calcium hydroxide dressing [10, 11, 22]. Moreover, the ultrasonically activated sodium hypochlorite was more effective in TAP removal than its use without activation [5]. Furthermore, PUI increases the efficacy of 17% EDTA in removal of calcium hydroxide residue [17].

Sonic irrigation was more efficient in the removal of antimicrobial dressing compared to traditional irrigation [23]. Also, it was used to remove debris more efficiently than traditionally, but its capacity was less than that of ultrasonic activation [24].

However, there was no statistically significant difference between ultrasonic and sonic activation in debridement of root canal isthmus [25]. Furthermore, sonic irrigation was more effective than ultrasonic activation in debris removal [26], showing a significant removal of smear layer than ultrasonic activation [27].

The results of the current study demonstrated a convergence of effectiveness of the ultrasonic and sonic activations in removal of DAP and $\text{Ca}(\text{OH})_2$ from three thirds of the canals, with no statistical difference. This is consistent with previous studies, which demonstrated the absence of a statistically significant difference between PUI and SI in dressing removal [18, 28, 29]. However, results of the current study disagree partially with results of Pabel *et al.* [12] and Wiseman *et al.* [21], who showed superiority of ultrasonic activation over sonic activation in calcium hydroxide removal from the root canals, whereas current results agree with the inability of complete residue removal using both of protocols.

These differences may be explained by different experience conditions or different anatomy of dental structures in addition to the difference of the studied material.

Although the ultrasonic activator operates at a constant high power and has effective acoustic streaming and cavitation, it cannot completely clean the apical third of the canal, as the file touches the canal walls, which limits its capacity [30]. Moreover, the sonic activator with a lower battery resource and a lower engine frequency would logically suffer from the same problem, which could be confusing.

Even though there is no ideal irrigation protocol that completely removes the entire antimicrobial dressing from root canals [4, 5, 9, 10, 12, 15, 16, 21, 23, 28, 30], the hybrid protocol in the current study was related to the least amount of residue.

Table 2. Mann-Whitney test results of removal ability of the two dressings

Irrigation protocol/Third/ Dressing type	Test value	p-value	Decision
Ultrasonic			
Coronal third			
DAP	−1.000	0.317	No significant difference
CA(OH) ₂			
Middle third			
DAP	−1.834	0.067	No significant difference
CA(OH) ₂			
Apical third			
DAP	−1.594	0.111	No significant difference
CA(OH) ₂			
Sonic			
Coronal third			
DAP	0	1.000	No significant difference
CA(OH) ₂			
Middle third			
DAP	−0.438	0.661	No significant difference
CA(OH) ₂			
Apical third			
DAP	−1.011	0.312	No significant difference
CA(OH) ₂			
Hybrid			
Coronal third			
DAP	0	1.000	No significant difference
CA(OH) ₂			
Middle third			
DAP	−0.503	0.615	No significant difference
CA(OH) ₂			
Apical third			
DAP	−0.175	0.861	No significant difference
CA(OH) ₂			

Finally, the hybrid activation of 17% EDTA was better than the ultrasonic and sonic activation of each one separately in the apical third. This can be explained by taking advantage of the combination of two methods in the dynamic field within a three-dimensional range as well as to the ability of the EDTA itself to chelate inorganic components, which agree with previous researches.

CONCLUSIONS

Hybrid activation showed predictable removal of residues of both dressings in the apical third, but there

is no protocol that can completely remove the dressing from the canal.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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