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ORIGINAL RESEARCH

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Effect of different activation methods on the intratubular penetration of $CaOH_2$ paste: Ex-vivo analysis by confocal laser scanning microscopy

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Abstract

The aim of this study was to evaluate the maximum penetration depth (MPD) and penetration area (PA) of $CaOH_2$ paste agitated by different methods and to measure the pH after performing these methods. Fifty-five mandibular premolars were divided into control, GL and 4 experimental groups, GEC, GXP, GEDDY and GI ($n = 11$), where the paste was agitated with Easy Clean, XP-endo Finisher, EDDY and Irrisonic, respectively. The canals were instrumented and irrigated with NaOCl. The paste was manipulated with propylene glycol and rhodamine B dye. Root sections were examined using confocal laser scanning microscopy. There was no difference between groups regarding PA ($p > 0.05$). At 5 mm, MPD was greater in GEC than in GL or GEDDY, and greater in GI than in GL ($p < 0.05$). At 2 mm, MPD was greater in GEC than in the other groups ($p < 0.05$). In conclusion, both Easy Clean and Irrisonic promoted deeper paste penetration and were associated with higher pH levels.

KEYWORDS

calcium hydroxide, confocal laser scanning microscope, molar, root canal medicaments, root canal treatment

INTRODUCTION

Endodontic treatment involves the use of chemical and mechanical agents to reduce the bacterial load within the root canal system (RCS), with the ultimate goal of curing, or preventing, pulp and periapical disease [1]. However, an intracanal medication may be indicated [2] to fight microorganisms capable of resisting the chemo-mechanical preparation, such as *Enterococcus faecalis* [3]. Furthermore, it should be kept in mind that no instrument is capable of touching all the canal walls, that irrigating solutions cannot always reach these microorganisms, even when agitated, and that these microorganisms are highly capable of penetrating dentinal tubules [4].

Currently, calcium hydroxide ($CaOH_2$) pastes are widely used as intracanal medications, particularly for their strong antibacterial properties. In addition to their contribution to interrupting resorption processes and dissolving organic tissue [5], they can raise the pH of the medium, thereby damaging the membrane of the microorganisms and limiting their respiratory support [6]. However, these medications must come into direct contact with the bacterial cells to perform these actions effectively, especially in the case of *Enterococcus faecalis* [3].

Different types of devices used for enhancing decontamination of the RCS by activating irrigating solutions have been tested, yielding improved treatment success rates [7–9]. Considering this successful application and the need to promote close contact between the $CaOH_2$ and the microorganisms in the RCS, Duarte et al. [10] and Arias et al. [11] investigated the effects of $CaOH_2$ paste agitation on dentinal tubule penetration, and observed high levels of penetration depth and calcium release.

De Almeida et al. [12] compared sonic and ultrasonic agitation methods regarding the degree of $CaOH_2$ paste penetration, and observed that the factor most significantly associated with this penetration was the vehicle used and not the agitation method per se. They concluded that the low surface tension of propylene glycol provided a more favourable flow of the paste into the dentinal tubules than distilled water. However, other agitation systems are currently available, such as Easy Clean (Easy Equipamentos Odontológicos), XP-endo Finisher (FKG Dentaire, La Chaux de Fonds, Switzerland) and EDDY (VDW), all of which are devices commonly used to agitate irrigating solutions during endodontic treatment or retreatment.

XP-endo Finisher (FKG Dentaire) is driven by an endodontic motor and has already proven effective in removing the smear layer by agitation of ethylenediaminetetraacetic acid (EDTA) [13]. EDDY (VDW) is a device capable of promoting sonic and ultrasonic vibrations, and its performance has proven superior to that of passive ultrasonic irrigation (PUI) [14]. Lastly, the Easy Clean instrument (Easy Equipamentos Odontológicos) features a flexible plastic tip designed to operate at low speed in reciprocating motion and has provided excellent results as a method of agitating irrigants to increase the cleanliness of the RCS, including isthmuses and flattened areas. The instrument has shown even more satisfactory results when used in continuous rotation [8].

The present study was premised on the potential benefits of (a) promoting further intratubular penetration of $CaOH_2$ paste, (b) providing this paste with a vehicle capable of ensuring optimal flow and (c) identifying the most effective agitation method to optimise its effects. To this end, it used confocal laser scanning microscopy (CLSM) to assess the maximum penetration depth (MPD) and penetration area (PA) of $CaOH_2$ paste in dentinal tubules, using propylene glycol as a vehicle, after agitation with XP-endo Finisher, Easy Clean, EDDY (sonic vibration) or Irrisonic instruments (ultrasonic vibration; Helse Ultrasonic). The pH level of the extraradicular medium was also measured after the agitation procedure, given that raising alkalinity can render the antibacterial action of the paste more effective. The null hypothesis considered was that there would be no difference among the agitation methods tested, regarding the MPD, PA and pH variables.

MATERIALS AND METHODS

Specimen selection

This study was approved by the local research ethics committee (approval no. 5.040.597). In addition, it was written according to the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines [15]. It was estimated that a minimum of 9 specimens per study group would be needed, based on the data from a previous study on the intratubular penetration of endodontic pastes [12] and on an effect size of 1.52, a type I error of 5%, a test power of 0.80 and a radius of 1. Therefore, the 11 teeth used in each group were considered sufficient and took into account the risk of specimen loss during the experiments. Sample size calculation was performed using the G*Power program v. 3.1 [16].

Fifty-five permanent mandibular premolars, extracted for unknown reasons, were obtained from the biorepository of the institution where the study was conducted and were selected based on radiographs taken in the buccolingual and mesiodistal directions. Only teeth with a single foramen and a straight canal (Type I in the Vertucci classification) [17] were included. Teeth with more than one canal, with any fractures, root cracks, resorptions (external or internal) or sharp curvatures were excluded. The selected specimens were stored in a 0.1% thymol solution until the beginning of the experiments.

Specimen preparation

The tooth crowns were sectioned with a carborundum disc (Dentorium-Labordental), and the roots were standardised at 12 mm, as measured with a digital calliper (Digimess). Each root canal was located and explored with a #10 K-type file (Dentsply Sirona,) and with the assistance of an operating microscope (DFVasconselos) under 8x magnification. Foraminal patency was maintained with a #15 K-type file, and root length was confirmed by introducing a #15 K-type file until its tip was just visible past the apical foramen. The working length (WL) was established 1 mm short of the apical foramen. The foramen was then sealed with utility wax to prevent any overflow of either the irrigant or paste.

Root canal preparation was performed by a single operator using the WaveOne Gold Medium instrument (35/0.06; Dentsply Sirona) driven by an endodontic motor (VDW Silver, VDW) in 'WaveOne All' mode and applying the movements recommended by the manufacturer up to the WL. At each 4 mm advancement of the instrument inside the canal, irrigation was performed with 5 ml of 2.5% sodium hypochlorite (NaOCl; Asfer) using a 5 ml special hypodermic syringe (Ultradent) and 30G NaviTip needle (Ultradent), totalling 15 ml of NaOCl per specimen. Patency was checked at each irrigation step with a #15 K-type file. All of the procedures were performed under 8x magnification.

After instrumentation, the specimens were irrigated according to the protocol described by Vivan et al. [9], whereby the root canal was flooded with 2 ml of 2.5% NaOCl, and the irrigant was agitated with an ultrasonic insert (Irrisonic E1; Helse Ultrasonic) activated by an ultrasound unit (Acteon-Satelec, Indaiatuba) set to operate at a power level of 20%, as recommended by the manufacturer. The insert was moved for 20 s in the cervicoapical direction, and the irrigant was refreshed between each time period; this cycle was performed 3 times. The same procedure was then performed using 2 ml of 17% EDTA (Biodynamics) and then again with 5 ml of saline solution. The canals were aspirated with an endodontic aspirator (Angelus) and then dried using a 0.36 mm diameter capillary tip (Ultradent) and #35 sterilised absorbent paper points (Dentsply Sirona).

Insertion and activation of the $CaOH_2$ paste

The amounts of $CaOH_2$ powder (Maquira), 0.1% rhodamine B dye (Sigma-Aldrich) and propylene glycol vehicle (Farmácia Posanga) were determined based on a study conducted by de Almeida et al. [12]. In all the study groups, the paste was manipulated at a proportion of 1 measuring scoop (from a Maxxion R glass ionomer cement set; FGM) of powder to 0.7 ml of the vehicle, as measured with an Eppendorf pipette (Eppendorf do Brasil). Approximately 0.01 g of the dye – an amount carried on the tip of a #5 dental explorer probe (SS White Duflex) – was added to the mixture, which was then manipulated on a glass plate with a #24 spatula so as to incorporate the dye into the paste completely. One specimen from each group was randomly set aside (www.random.org) for the next step of pH measurement, in which no dye was added to the mixture. The 55 specimens were then randomly divided (www.random.org) into 5 groups ($n = 11$) according to the insertion and agitation procedure employed. The same operator, blinded to specimen grouping, performed all the $CaOH_2$ insertion and agitation procedures, as described below.

- GL (Control): the $CaOH_2$ paste was manipulated and inserted with a Lentulo drill size 1 coupled to a low-speed handpiece (KaVo Dental, Berlin, Germany) up to 2 mm short of the apical foramen, making light in-and-out movements until the canal was completely filled.

- GEC: the $CaOH_2$ paste was inserted as in GL and then agitated with the Easy Clean instrument, coupled to a low-speed handpiece set to operate in continuous rotation at 20000 rpm and inserted up to 2 mm short of the foramen, making light in-and-out movements for 30 s.

- GXP: the $CaOH_2$ paste was inserted as in GL and then agitated with the XP-endo Finisher instrument coupled to an endodontic motor (VDW Silver) set to operate in continuous rotation, with 1 N torque, and at 800 rpm, making light in-and-out movements for 30 s, following the manufacturer's recommendations.

- GEDDY: the $CaOH_2$ paste was inserted as in GL and then agitated with the EDDY instrument, driven by a pneumatic sonic device (Airscaler Sonic Borden 2000 N; Kavo, Biberach, Germany) set to operate at 6000 Hz; the instrument was moved in the cervicoapical direction for 30 s.

- GI: the $CaOH_2$ paste was inserted as in GL and then agitated with an Irrisonic insert coupled to an ultrasound unit (Acteon-Satelec) set to operate with ultrasonic vibration at 20% power; the insert was moved in the cervicoapical direction for 30 s.

Measurement of the pH level

One specimen from each group was randomly set aside for the pH measurement step and prepared in the same way as the others; however, the dye was not mixed with the $CaOH_2$ paste, to prevent interference with the pH level. After inserting and agitating the paste following the procedure stipulated for each group, the specimen's root was sealed with a light-curing resin (Z350; 3 M do Brasil), submerged in a bottle with 20 ml of distilled water at 37°C and kept therein for 15 days; the commonly recommended period of time between endodontic treatment sessions when using a viscous vehicle. After this period, the pH of the extraradicular medium was measured using a pH meter (Analyser Instrumentação Analítica) previously calibrated according to constant measurements of known buffer solutions with pH levels of 4, 7 and 12.

CLSM image acquisition and analysis

The specimens with dyed paste were cross-sectioned with a microtome (Isomet 1000; Buehler), at low speed (200 rpm) under constant cooling, to produce 1 mm thick sections from the levels of 2 and 5 mm short of the foramen. These sections were washed in distilled water, dried with a gentle air jet, sanded and polished with a polisher using 800, 1000 and 1200 grit sandpaper and then fixed on glass slides to be analysed under a confocal laser scanning microscope (Leica TCS SPE; Leica Microsystems).

Image acquisition was performed using a 5x objective, with a field of view of 2 x 2 mm and a laser wavelength of 543 nm to excite the rhodamine B dye. A single optical section was used, approximately 10 µm below the surface. The spectral detector was set to operate in the 545–740 nm range.

MPD and PA measurements were performed using Image J software (U. S. National Institutes of Health). The program was calibrated by using the 'spatial calibration' tool, and by drawing a straight line over the scale bar displayed in the image (500 µm) projected by the confocal microscope software. The MPD was measured by dividing each image into quadrants, and the 'distance' tool was used in each quadrant to determine the measurement (in µm) between the two points delimiting the depth of paste penetration. The procedure used a starting point on the canal wall, and an end point defined as the point farthest from the canal wall along with the course of the tubule towards the outer root surface. The PA was also measured in each quadrant by using colour and saturation tools to distinguish the area (in µm^2) penetrated by the paste from the area of the remainder of the image. The values were then tabulated and statistically evaluated.

Statistical analysis

Initially, the mean value obtained in each quadrant was calculated. The Shapiro–Wilk normality test was then performed to assess the data distribution pattern, which revealed a normal distribution. The MPD and PA data obtained for the study groups in each region of interest (2 or 5 mm short of the foramen) were compared using the Kruskal-Wallis test followed by Dunn's test for multiple comparisons, whereas the data obtained for the regions of interest (2 and 5 mm) within each study group were compared using the Mann–Whitney test. The extraradicular pH data were compared using the Kruskal-Wallis test and the Mann–Whitney post-test. Both IBM SPSS (26.0, IBM Corporation) and GraphPrism (9.2.0, GraphPad Software) software was used in the analyses. The level of significance used was 5%.

RESULTS

Figure 1 contains representative CLSM images of the specimens from the study groups, taken at 2 and 5 mm from the foramen, after the insertion and agitation of the CaOH$_2$ paste. There was no significant difference among the groups with respect to PA, at either root level ($p > 0.05$; Table 1). The intragroup comparisons revealed that PA values were significantly greater at 5 mm than at 2 mm from the foramen, in all the study groups ($p < 0.05$; Table 2).

At 5 mm, the MPD was significantly greater in GEC than in GL or GEDDY ($p < 0.05$) and also greater in GI than in GL ($p < 0.05$; Table 1). At 2 mm, MPD was significantly greater in GEC than in GL, GXP or GEDDY ($p < 0.05$; Table 1). The intragroup comparisons revealed that MPD values were significantly greater at 5 mm than at 2 mm from the foramen, in all the study groups ($p < 0.05$; Table 2).

The pH values in GL were significantly lower than in the other groups ($p < 0.05$); however, the post-test revealed that they were similar to those observed in GXP ($p > 0.05$). Those observed in GEC were higher than those observed in the other groups; however, the post-test revealed that they were similar to those observed in GI ($p > 0.05$; Table 3).

DISCUSSION

According to Stuart et al. [18], endodontic treatment failure can be directly linked to microorganisms that resist the chemomechanical preparation of the root canal, especially those located in areas of higher anatomical complexity. In 1976, Sundqvist [19] associated the presence of bacteria inside the RCS with pulp and periapical pathologies, and some of these microorganisms proved capable of invading and colonising the dentinal tubules [18]. Behnen et al. [20] pointed out that RCS infection or reinfection are linked to the ability of microorganisms to penetrate the dentinal tubules. As a result, applying a technique capable of improving the intratubular penetration of intracanal medication came to be considered a potential risk reduction factor for infection. Furthermore, Wang & Hume [21] showed that low penetration of CaOH$_2$ into dentin was related to the buffering action of this tissue. Therefore, performing a paste agitation technique that could promote higher levels of MPD and PA could, in turn, lead to an increase in dentin pH, hence rendering the environment unfavourable to bacterial multiplication.

The null hypothesis was partially rejected, since there was a significant difference among the groups with respect to MPD and pH values, corroborating the studies conducted by Duarte et al. [10], Arias et al. [11], and de Almeida et al. [12], who observed a significant increase in these variables when the paste was agitated by Easy Clean at low speed [8] and by the ultrasonic method. In contrast, no significant difference among the groups was found with respect to PA in the present study.

CLSM has been used to analyse hard tissue or the penetration of substances associated with dyes into hard tissue, such as intracanal medications and sealers [22]. This method does not require specimen dehydration or

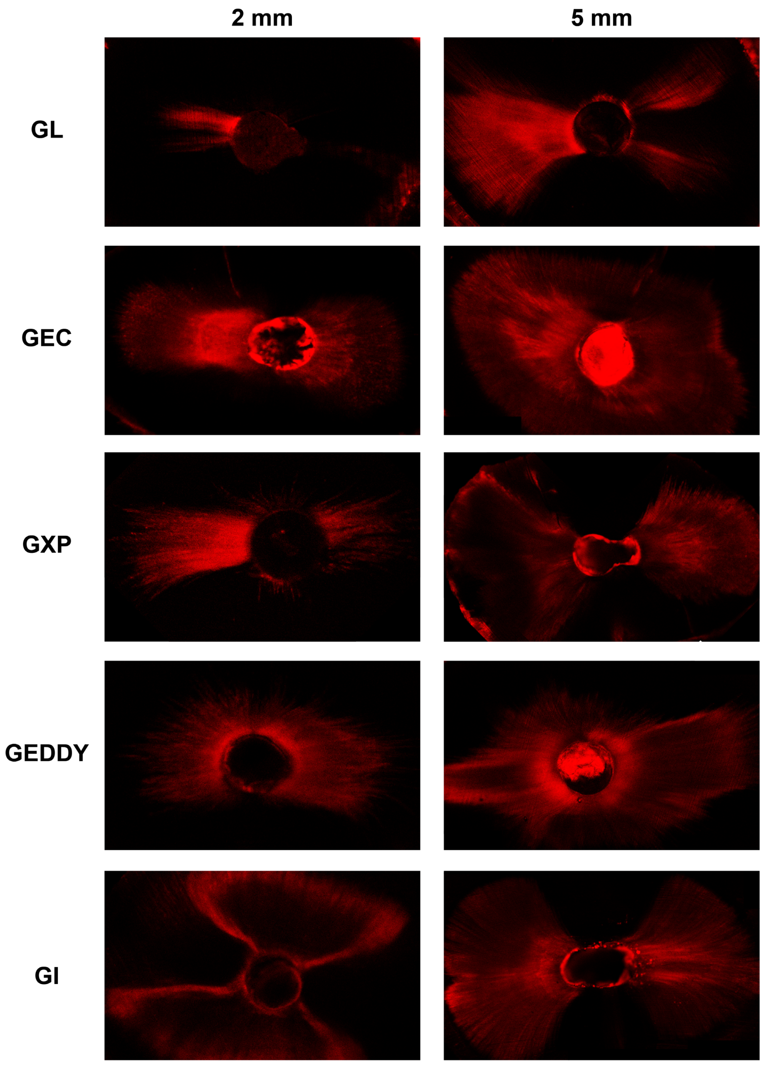

FIGURE 1 Representative images of specimens from the study groups obtained with a confocal laser scanning microscope, at the levels of 2 and 5 mm from the root foramen. GL: insertion of the $CaOH_2$ paste with a Lentulo spiral, without agitation (control); GEC: insertion with Lentulo spiral followed by agitation with Easy Clean; GI: insertion with Lentulo spiral followed by agitation with Irrisonic; GXP: insertion with Lentulo spiral followed by agitation with XP-endo Finisher; GEDDY: insertion with Lentulo spiral followed by agitation with EDDY

promote any change to the materials within the canals and also allows visualisation of the degree of filling of the dentinal tubules [23]. In the present study, a small amount of rhodamine B associated with the $CaOH_2$ paste was used to allow visualisation of intratubular penetration, and this amount of dye has been shown not to interfere with the physicochemical properties of the paste [24]. A Lentulo spiral driven at low speed was used to carry the paste into the root canal, an instrument still widely used for its proven satisfactory filling results [25]. The use of mandibular premolars allowed specimen standardisation, since these teeth commonly have a single root, with a straight and single canal [26]. In contrast, molars may be more difficult to standardise, hence potentially leading to biased results [27].

The vehicle used to manipulate and carry the $CaOH_2$ into the canal may be considered a critical factor in the application of this medication, because of its direct influence on the degree of flow and penetration of the paste. In the present study, intratubular penetration was observed in all the groups, likely owing to the low surface tension of the vehicle, corroborating the study conducted by de Almeida et al. [12], who observed that propylene glycol was associated with better paste penetration results than distilled water, at the level of 5 mm from the foramen.

Arias et al. [10] compared the intratubular MPD of the paste in bovine teeth with and without ultrasonic agitation, and observed a significant MPD increase when this method was used, confirming the results of the present study. Likewise, Duarte et al. [10] and de Almeida et al. [12] obtained superior spreading results when the paste was activated by an ultrasonic method.

Previous studies have shown that the minimum period required for $CaOH_2$ to start exerting its antimicrobial action is 7 days, and that this period may vary depending on the vehicle used [3]. Kim & Kim [28] demonstrated that longer $CaOH_2$ permanence times within the canal can render the medium more alkaline, thus confirming the results of the present study. Camargo et al. [4] studied different periods of $CaOH_2$ paste maintenance inside the canals, and, based on their results, recommended that the medication be maintained for at least 14 days.

TABLE 1 Comparison of the study groups regarding the values for penetration area (PA, in μm^2) and maximum penetration depth (MPD, in μm) of the $CaOH_2$ paste

		Penetration area (PA)		Maximum penetration depth (MPD)	
Comparison	**Root level (short of the foramen)**	**Mean rank difference**	***p*-value**	**Mean rank difference**	***p*-value**
GL vs. GEC	2 mm	−12.50	0.5519	−51.78	0.0006 AB
	5 mm	−13.20	0.4289	−53.00	0.0004 AB
GL vs. GXP	2 mm	2.500	< 0.9999	−2.575	< 0.9999
	5 mm	−4.700	< 0.9999	−17.23	< 0.9999
GL vs. GEDDY	2 mm	1.600	< 0.9999	−8.300	< 0.9999
	5 mm	−2.900	< 0.9999	−10.44	< 0.9999
GL vs. GI	2 mm	−7.100	< 0.9999	−30.73	0.1760
	5 mm	−6.700	< 0.9999	37.84	0.0346 AB
GEC vs. GXP	2 mm	15.00	0.2140	49.20	0.0014 AB
	5 mm	8.500	< 0.9999	35.78	0.0571
GEC vs. GEDDY	2 mm	14.10	0.3055	43.48	0.0078 AB
	5 mm	10.30	< 0.9999	42.56	0.0101 AB
GEC vs. GI	2 mm	5.400	< 0.9999	21.05	< 0.9999
	5 mm	6.500	< 0.9999	15.16	< 0.9999
GXP vs. GEDDY	2 mm	−0.9000	< 0.9999	5.725	< 0.9999
	5 mm	1.800	< 0.9999	6.788	< 0.9999
GXP vs. GI	2 mm	−9.600	< 0.9999	−28.15	0.2963
	5 mm	−2.000	< 0.9999	−20.61	< 0.9999
GEDDY vs. GI	2 mm	−8.700	< 0.9999	−22.43	0.8315
	5 mm	−3.800	< 0.9999	−27.40	0.3425

Note: Different uppercase letters indicate a statistically significant difference between the study groups (Kruskal-Wallis test followed by Dunn's test for multiple comparisons, $p < 0.05$), in each of the root levels evaluated (2 or 5 mm short of the foramen).

Abbreviations: GEC, agitation with Easy Clean; GEDDY, agitation with EDDY; GI, agitation with Irrisonic; GL, insertion with a Lentulo spiral, without agitation (control); GXP, agitation with XP-endo Finisher.

TABLE 2 Intragroup comparison of the root levels (2 and 5 mm short of the foramen) regarding the values for penetration area (PA, in μm^2) and maximum penetration depth (MPD, in μm) of the $CaOH_2$ paste

	Penetration area (PA)		Maximum penetration depth (MPD)	
Group	**Sum of ranks (2 mm vs. 5 mm)**	***p*-value**	**Sum of ranks (2 mm vs. 5 mm)**	***p*-value**
GL	56 vs. 154	<0.0001 AB	834 vs. 2406	<0.0001 AB
GEC	64 vs. 146	0.0011 AB	1145 2095	<0.0001 AB
GXP	72 vs. 138	0.0115 AB	996 vs. 2244	<0.0001 AB
GEDDY	74 vs. 136	0.0185 AB	1081 vs. 2159	<0.0001 AB
GI	71 vs. 139	0.0089 AB	1130 vs. 2110	<0.0001 AB

Note: Different uppercase letters indicate a statistically significant difference between the root levels, in each of the study groups (Mann–Whitney test, $p < 0.05$).

Abbreviations: GEC, agitation with Easy Clean; GEDDY, agitation with EDDY; GI, agitation with Irrisonic; GL, insertion with a Lentulo spiral, without agitation (control); GXP, agitation with XP-endo Finisher.

In the present study, higher levels of penetration and filling were observed in the cervical root third (5 mm short of the foramen), probably due to the larger calibre of dentinal tubules, and larger diameter of the root canal itself in this region, which facilitates $CaOH_2$ paste agitation. This result was also observed elsewhere [11, 12].

According to Heward & Sedgley [5] and Tronstad et al. [29], penetration of the paste into dentinal tubules

TABLE 3 Extraradicular pH values observed in the study groups

Group	pH	*p*-value
GL	9.74 [A]	<0.001
GEC	11.57 [B]	
GXP	9.93 [AC]	
GEDDY	10.16 [CD]	
GI	10.47 [BD]	

Note: Different uppercase letters indicate a statistically significant difference between the study groups (Kruskal-Wallis test and Mann–Whitney post-test, $p < 0.05$).

Abbreviations: GEC, agitation with Easy Clean; GEDDY, agitation with EDDY; GI, agitation with Irrisonic; GL, insertion with a Lentulo spiral, without agitation (control); GXP, agitation with XP-endo Finisher.

increases the alkalinity of the external root surface, contributing to halt resorption processes and eliminate microorganisms that are resistant to chemomechanical preparation. Furthermore, according to these authors, activating the paste would not only improve its intratubular penetration, but would also contribute to improve its penetration into the most inaccessible ramifications of the RCS, capable of harbouring resistant microorganisms. In their research, de Almeida et al. [12] did not assess the pH of the specimens, an important factor to ensure disinfection of the RCS. Conversely, assessment of the pH value in the present study showed that agitation performed with both ultrasound and Easy Clean raised the pH level and potentially led to increasing the antimicrobial action of the paste, as described by Arias et al. [11]. Higher pH levels can be attributed to paste agitation, in that this procedure can promote an increase in the dissociation of $CaOH_2$ into calcium ions. Accordingly, Duarte et al. [10] demonstrated that paste agitation had a greater influence on increasing pH than the vehicle itself.

According to Duque et al [8], the good results obtained with Easy Clean are linked to the high rotation speed produced during its activation and to the fact that agitation occurs along with the entire length of the instrument, promoting a better distribution of the paste inside the RCS. On the other hand, sonic and ultrasonic devices produce acoustic microstreaming, a phenomenon that can push the paste against the canal walls, causing it to penetrate dentinal tubules and canal ramifications [9], whereas the XP-endo Finisher instrument performs a helical movement that enables it to contact areas left untouched by instrumentation, hence carrying the paste to these areas [13]. In the present study, however, neither the sonic nor the XP-endo Finisher system performed as effectively as Easy Clean in the most apical regions of the canal, reinforcing the hypothesis posited by Duque et al [8].

The results of the present study, if confirmed, represent a promising prospect for clinical endodontics, in that they demonstrate the use of established irrigant agitation technologies to achieve a significant therapeutic benefit; however, the study was limited to evaluating $CaOH_2$ paste activation in single and straight canals of mandibular premolars, hence teeth with a less challenging anatomy. Future studies should be carried out to assess the penetration of this medication in teeth with sharper curvatures, such as mandibular molars, considering that not all of the instruments tested (or their effects) are capable of overcoming these anatomical obstacles.

In addition, future investigations should ascertain whether the benefits of enhancing intratubular penetration of the intracanal medication by using the methods tested herein will in fact result in higher rates of clinical success, especially in cases where there is a periapical lesion. This question is particularly relevant when considering that bacteria residing in dentinal tubules can be entombed by root canal filling material and, as a result, be unable to provide the virulence needed to effectively threaten treatment outcomes. Furthermore, according to this rationale, the intracanal medication penetrating the tubules could, in turn, compromise the tubular penetration of sealers, hence potentially compromising the desired entombing effect.

In conclusion, and within the limitations of the methodology used in this study, the agitation produced by the Easy Clean instrument in continuous rotation, and that produced by the Irrisonic insert at an ultrasonic frequency, promoted significantly higher levels of intratubular MPD of the $CaOH_2$ paste than the other methods tested. In addition, agitation with Easy Clean and Irrisonic was associated with a significantly higher pH of the extraradicular medium than with the other methods.

AUTHOR CONTRIBUTIONS

J.P.G.T. and C.E.S.B. contributed substantially to the conception and design of the study, and to the acquisition, analysis and interpretation of the data; he also drafted the text and revised it critically for important intellectual content. C.F.M.S. contributed substantially to the analysis and interpretation of the data; she also revised the text critically for important intellectual content. T.B.Y. contributed substantially to the design of the study, and to the analysis, and interpretation of the data; she also revised the text critically for important intellectual content. G.G.P. contributed substantially to the analysis, and interpretation of the data; she also revised the text critically for important intellectual content. M.A.H.D. contributed substantially to the conception and design of the study, and to interpretation of the data; he also revised the text critically for important intellectual content. R.R.V. contributed substantially to the analysis and interpretation of the data; he also revised the text critically for important intellectual content. A.S.M. contributed substantially to the conception and design of the study, and to the analysis, and interpretation of the data; he also revised the text critically for important intellectual content. All authors approved the final version of the manuscript to be published, and agreed to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of it were appropriately investigated and resolved.

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None to declare

CONFLICT OF INTEREST

No conflict of interest to disclose

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REFERENCES

1. Donyavi Z, Ghahari P, Esmaeilzadeh M, Kharazifard M, Yousefi-Mashouf R. Antibacterial efficacy of calcium hydroxide and chlorhexidine mixture for treatment of teeth with primary endodontic lesions: a randomized clinical trial. Iran Endod J. 2016;11:255–60.
2. Nair P. On the causes of persistent apical periodontitis: a review. Int Endod J. 2006;39:249–81.
3. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J. 1991;24:119–25.
4. Camargo CH, Bernardineli N, Valera MC, de Carvalho CAT, de Oliveira LD, Menezes MM, et al. Vehicle influence on calcium hydroxide pastes diffusion in human and bovine teeth. Dent Traumatol. 2006;22:302–6.
5. Heward S, Sedgley CM. Effects of intracanal mineral trioxide aggregate and calcium hydroxide during four weeks on pH changes in simulated root surface resorption defects: an in vitro study using matched pairs of human teeth. J Endod. 2011;37:40–4.
6. Ran S, Wang J, Jiang W, Zhu C, Liang J. Assessment of dentinal tubule invasion capacity of Enterococcus faecalis under stress conditions ex vivo. Int Endod J. 2015;48:362–72.
7. van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J. 2007;40:415–26.
8. Duque JA, Duarte MAH, Canali LC, Zancan RF, Vivan RR, Bernardes RA, et al. Comparative effectiveness of new mechanical irrigant agitating devices for debris removal from the canal and isthmus of mesial roots of mandibular molars. J Endod. 2017;43:326–31.
9. Vivan RR, Duque JA, Alcalde MP, Só MV, Bramante CM, Duarte MA. Evaluation of different passive ultrasonic irrigation protocols on the removal of dentinal debris from artificial grooves. Braz Dent J. 2016;27:568–72.
10. Duarte MAH, Balan NV, Zeferino MA, Vivan RR, Morais CAH, Tanomaru-Filho M, et al. Effect of ultrasonic activation on pH and calcium released by calcium hydroxide pastes in simulated external root resorption. J Endod. 2012;38:834–7.
11. Arias MP, Maliza AG, Midena RZ, Graeff MS, Duarte MA, Andrade FB. Effect of ultrasonic streaming on intra-dentinal disinfection and penetration of calcium hydroxide paste in endodontic treatment. J Appl Oral Sci. 2016;24:575–81.
12. de Almeida BM, de Oliveira KV, dos Santos VR, da Silva WJ, FSF T, Baratto-Filho F, et al. Effect of vehicle and agitation methods on the penetration of calcium hydroxide paste in the dentinal tubules. J Endod. 2020;46:980–6.
13. Zand V, Mokhtari H, Reyhani MF, Nahavandizadeh N, Azimi S. Smear layer removal evaluation of different protocols of Bio Race file and XP-endo Finisher file in corporation with EDTA 17% and NaOCl. J Clin Exp Dent. 2017;9:e1310–4.
14. Donnermeyer D, Wyrsh H, Bürklein S, Schäfer E. Removal of calcium hydroxide from artificial grooves in straight root canals: sonic activation using EDDY versus passive ultrasonic irrigation and XP-endo Finisher. J Endod. 2019;45:322–6.
15. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF Jr, et al. PRILE 2021 guidelines for reporting laboratory studies in endodontology: a consensus-based development. Int Endod J. 2021;54:1482–90.
16. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41:1149–60.
17. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol. 1984;58:589–99.
18. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32:93–8.
19. Sundqvist G. Bacteriological studies of necrotic dental pulps, Umeå. Sweden: Dept. of Oral Microbiology, University of Umeå; 1976.
20. Behnen MJ, West LA, Liewehr FR, Buxton TB, McPherson JC. Antimicrobial activity of several calcium hydroxide preparations in root canal dentin. J Endod. 2001;27:765–7.
21. Wang JD, Hume WR. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. Int Endod J. 1998;21:17–26.
22. van der Veen MH, ten Bosch JJ. Autofluorescence of bulk sound and in vitro demineralized human root dentin. Eur J Oral Sci. 1995;103:375–81.

23. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P, et al. Microscopy investigations. techniques, results, limitations. Am J Dent. 2000;13:3D–18D.
24. Patel DV, Sherriff M, Ford TR, Watson TF, Mannocci F. The penetration of RealSeal primer and Tubliseal into root canal dentinal tubules: a confocal microscopic study. Int Endod J. 2007;40:67–71.
25. Torres CP, Apicella MJ, Yancich PP, Parker MH. Intracanal placement of calcium hydroxide: a comparison of techniques, revisited. J Endod. 2004;30:225–7.
26. Cleghorn BM, Christie WH, Dong CC. The root and root canal morphology of the human mandibular first premolar: a literature review. J Endod. 2007;33:509–16.
27. Wolf TG, Wentaschek S, Wierichs RJ, Briseño-Marroquín B. Interradicular root canals in mandibular first molars: a literature review and ex vivo study. J Endod. 2019;45:129–35.
28. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review – Part I. In vitro studies Restor Dent Endod. 2014;39:241–52.
29. Tronstad L, Andreasen JO, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root canal filling with calcium hydroxide. J Endod. 1981;7:17–21.

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