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Calcium hydroxide diffusion after agitation of endodontic irrigants: an *ex vivo* study

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Aim: To compare the influence of two methods of agitation of endodontics irrigants, by diffusion of calcium hydroxide [$Ca(OH)_2$] through the dentinal tubules, measuring the pH of the medium where they were kept. **Methods:** Twenty mandibular incisors were prepared using a WaveOne Gold Large file, in a reciprocating movement, and then divided into (n = 10): gutta-percha cone (GPC) or Easy Clean system (ECS) agitation of 1% sodium hypochlorite and 17% ethylenediaminetetraacetic acid. The specimens were filled with $Ca(OH)_2$ paste, placed in flasks with 4 mL of deionized water, and stored in an incubator. The pH was read using a digital pH meter immediately after storage (T0), after 7 (T1), 14 (T2), 21 (T3), and 35 (T4) days. **Results:** Statistical difference between groups was observed regardless of the day pH was measured ($p < 0.01$). From T2 on, ECS presented higher pH values in comparison with GPC, with significant difference ($p < 0.01$). **Conclusion:** Agitation of endodontic irrigants with ECS enhances the $Ca(OH)_2$ diffusion, providing higher pH values, from the 14th day on, when compared with GPC.

Keywords: Calcium hydroxide. Endodontics. Root canal irrigants. Sodium hypochlorite.

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Introduction

Cleaning and shaping can be considered the most important step of endodontic treatment, because, in addition to preparing the site for three-dimensional obturation, it eliminates bacterial infection of the root canal system[1].

Root canal anatomical complexities, including curvatures, isthmuses, lateral canals, apical ramifications, and recesses of oval-shaped, C-shaped, or flattened canals, may be challenging for endodontic therapy[2]. In these cases, the chemical effects of irrigants can take on their main role, especially in antisepsis in necrotic teeth and teeth with failed root canal treatments[3]. Moreover, irrigants as sodium hypochlorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA) can penetrate mechanically inaccessible areas, dissolving organic tissues and removing smear layers, being essential in endodontic treatment.

NaOCl is an acid solution (pH ≅ 5,0) commonly used in Endodontics, because it has a wide antimicrobial spectrum and it is capable of inhibiting or deactivating some bacterial enzymes through the formation of reactive chlorine, which causes irreversible oxidation of the sulfhydryl group of the bacterial enzymes[4,5]. Although NaOCl eliminates microorganisms[6], ethylenediaminetetraacetic acid (EDTA – pH ≅ 12) is used because of its capacity to dissolve the inorganic component of the smear layer[5,7].

The antibacterial effect, achieved by endodontic treatment, is more likely affected by the degree of the penetration of irrigants to scavenge bacteria residing deeply inside infected dentinal tubules than by the instrumentation of the root canal system, once the shaping protocol revealed deficient debridement and areas untouched by both, the manual files and rotary or reciprocating instruments[8]. Due to these limitations, in recent years, research on root canal irrigation quality and efficiency has focused on irrigants with better cleaning and antibacterial activity as a necessary complement to mechanical preparation[9]. Also, it is important to mention the influence of endodontic irrigants on sealer penetration into the dentinal tubules. NaOCl, when compared to other irrigant solutions, promoted more uniform sealer penetration[10].

Root canal irrigants reduce the number of microorganisms within superficial layers of root dentine, however, bacteria more deeply embedded within tubules often remain unaffected[11] and may contribute to persistent periradicular disease[12]. This barrier may be overcome through the use of irrigant activation techniques, allowing deeper areas of the dentine tubules to be reached[5,13]. The main techniques for this purpose are basically divided between manual techniques or use of a device[14], indicating that the latter has superior results[15,16]. In this context, the Easy Clean system - ECS (Easy Equipamentos Odontológicos, Belo Horizonte, MG, Brazil) is a device similar to a rotary endodontic instrument; its active part is in the shape of an "aircraft wing" and it is recommended for promoting endodontic irrigant agitation[17,18].

Thus, for eliminating microorganisms, the use of calcium hydroxide [$Ca(OH)_2$] paste is established as a form of intracanal medication, complementary to irrigation. With a pH of 12.5 to 12.8, its main antibacterial actions, tissue dissolution, inhibition of tooth resorption and induction of tissue deposition, result from the dissociation of calcium (Ca^{2}+) and hydroxyl (OH^{-}) ions[19]. This dissociation allows the ions to diffuse through the dentinal tubules[20]. For antibacterial action to occur within the dentinal tubules, the ionic diffusion of $Ca(OH)_2$ must be greater than the buffering capacity of the dentine, and the pH levels must be enough to eliminate microorganisms[19]. For this reason adequate prior cleaning with irrigants is essential.

Another important factor refers to the vehicles used to manipulate $Ca(OH)_2$ paste. The vehicles for $Ca(OH)_2$ powder aggregate are primarily of three types: aqueous, viscous, and oily. The high molecular weights of the vehicles minimize the dispersion of the materials in the tissues and retain the paste in the desired area for a longer duration. With respect to viscosity, the lower the viscosity, the higher the rate of ion dissociation, as evident with aqueous vehicles[21], that generate a higher ionic dissociation speed, while the opposite occurs with viscous vehicles[22].

Based on the presented, given that irrigant agitation techniques may have some impact on $Ca(OH)_2$ penetration through the dentinal tubules, this study aimed to compare manual use of GPC with ECS with regard to irrigant activation, measured by the pH values in the deionized water. The null hypothesis was that there would be no significant difference in pH values.

Material and Methods

This *ex vivo* study was approved by the Human Research Ethics Committee (no. 4.260.956). Sample size calculation used data from previous studies as a reference[11]. The power observed in the sample was calculated considering $\alpha = 5\%$ and the rejection of the null hypothesis through the ANOVA test, which resulted in a power value greater than 99%. Twenty mandibular incisors, with a single root canal, were selected from the Dental School's tooth bank.

All procedures were performed by the same operator, an endodontist, with 20 years of experience.

The inclusion criteria were: teeth with almost straight roots with similar size and shape (standardized using Cone Beam Computed Tomography – CBCT), completely closed apices, and absence of previous root canal treatment. The exclusion criteria were: presence of caries in the root and presence of visible fracture lines in the root. Calcified canals were replaced to maintain the sample (n = 20).

The method for ion diffusion analysis was adapted from Batista et al.[23]. Teeth were sectioned with rotating carborundum discs (Dentorium International, New York, NY, USA) using a low-speed handpiece at 14 mm from the apex. Thereafter, the specimens were stored in 0.9% saline solution (Eurofarma, São Paulo, SP, Brazil) at 9 ^{o}C for further procedures.

Initial canal exploration was performed with manual #10 K-files (Dentsply Sirona, Ballaigues, Switzerland), Proglider files (Dentsply Sirona, Ballaigues, Switzerland), syringe, and NaviTip FX irrigation needle (Ultradent Products Inc., South Jordan, UT, USA) containing 0.9% saline solution to verify the presence of a single root canal and to achieve apical patency. Working length (WL) was set at 13 mm for all specimens. Root canal preparation was performed using a WaveOne Gold Large file (Dentsply Sirona, Ballaigues, Switzerland) fitted to a VDW Silver motor (VDW, Munich, Germany) in a reciprocating movement.

The specimens were washed in deionized water. Then, the apices were closed using composite resin (VH Opallis; FGM, Joinville, SC, Brazil). The specimens were then divided into two groups (n = 10):

- GPC: 1 mL of 1% NaOCl was applied at the entry of the canal using a syringe and NaviTip FX irrigation needle (Ultradent Products Inc., South Jordan, UT, USA). The solution was manually activated in an up-and-down motion for 30 seconds using a #25 GPC (Dentsply Sirona, Ballaigues, Switzerland) at the WL. Subsequently, the canals were irrigated with 17% EDTA (Biodinâmica, Ibiporã, PR, Brazil) and agitated as described above for 30 seconds. 1 mL of 1% NaOCl was replaced at the entry of the canal and activated manually for a further 30 seconds.

- ECS: the procedure was similar to that used in the previous group, but activation was performed with ECS in continuous motion using an electric device at approximately 900 rpm and 2-Newton centimeters (Ncm) of torque (DForce 1000 Endo, Dentflex, Ribeirão Preto, SP, Brazil), at 2 mm from the WL.

After preparation the canals were dried with #25 paper points (Tanariman Industrial Ltda., Manacapuru, AM, Brazil), and filled with UltraCal using a syringe and a NaviTip FX needle (Utradent Products Inc., South Jordan, UT, USA). The access cavities were sealed with composite resin. The specimens were placed in flasks with 4 mL of deiozined water, and stored in an incubator with 100% humidity at 36 ^{o}C for up to 35 days.

The pH of the deionized water was read using a digital pH meter device (Quimis, Diadema, SP, Brazil) immediately after storage (T0), after 7 (T1), 14 (T2), 21 (T3), and 35 (T4) days. Prior calibration was performed using buffer solutions with acetic acid (pH 4.0) and sodium acetate (pH 7.0). For each measurement, the pH meter electrode was rinsed with deionized water and dried with absorbent paper to eliminate residues that might cause interference.

The data were analyzed using Stata/SE v.14.1 statistical software (StataCorp LLC., College Station, TX, USA).

The Kolmogorov-Smirnov test at 0.05 level showed that the samples were normally distributed ($p > 0.05$), allowing differences in mean pH values to be assessed. Two-Way Analysis of Variance (Two-Way ANOVA) tested the difference between the mean pH values of GPC and ECC, and also between the days the pH readings were taken. When Two-Way ANOVA indicated a difference between the variables ($p < 0.05$), the Tukey HSD (Honestly Significant Difference) test was performed for homogeneous variances, or the Games-Howell test was used for heterogeneous variances, both for multiple comparisons. Levene's test was used to compare the homogeneity of variance. The level of all tests was set at 0.05.

Results

Regardless of the day on which pH was measured, a statistically significant difference ($p < 0.01$) occured between the mean pH values of the two groups (Table 1).

Table 2 shows the statistical difference between each method at different observation periods (T). The highest pH value was observed at T4, with means and standard deviations for GPC and ECS of 7.28 (± 0.09) and 7.65 (± 0.08), respectively.

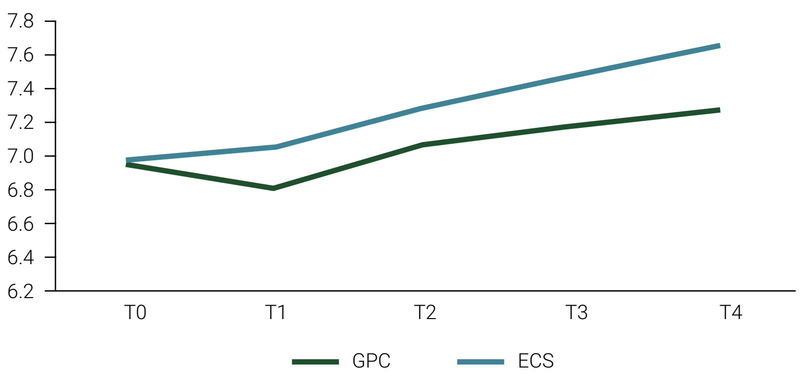
From T2 on, GPC and ECS presented an increase in mean pH values, but the ECS mean (7.29 ± 0.16) differed statistically from the GPS mean ($p < 0.01$). This difference was also found at T3 (7.47 ± 0.13) and T4 (7.65 ± 0.08) (Table 2).

Table 1. Mean pH values following each agitation technique, regardless of the observation periods.

Group	n	Mean (SD)	95%CI	
			Lower limit	Upper limit
GPC	50	7.05 (0.24)a	6.98	7.12
ECS	50	7.29 (0.30)b	7.20	7.38

Note: GPC, manual activation using gutta-percha cones; ECS, Easy Clean activation; SD, standard deviation; 95%CI, 95% confidence interval. Mean values followed by different letters indicate significant difference ($p < 0.05$).

Figure 1 shows the increase in mean pH values according to the technique and time. Higher mean pH values were found for ECS. The difference increased and was statistically significant ($p < 0.01$) from T2 onwards (Table 2).



Note: GPC, manual activation using gutta-percha cones; ECS, Easy Clean activation; T0, immediately after storage; T1, after 7 days; T2, after 14 days; T3 after 21 days; T4 after 35 days.

Figure 1. Mean pH values according to technique and time.

Table 2. Mean pH values in different observation periods (T) and agitation technique.

Group x T	n	Mean (SD)	95%CI	
			Lower limit	Upper limit
GPC x T0	10	6.95 (0.19)a,b	6.81	7.09
GPC x T1	10	6.81 (0.33)a	6.57	7.05
GPC x T2	10	7.07 (0.13)a,b	6.98	7.16
GPC x T3	10	7.17 (0.08)b,c	7.11	7.23
GPC x T4	10	7.28 (0.09)c,d	7.20	7.35
ECS x T0	10	6.98 (0.27)a,b	6.78	7.17
ECS x T1	10	7.06 (0.19)a,b,c	6.92	7.19
ECS x T2	10	7.29 (0.16)c,d	7.17	7.40
ECS x T3	10	7.47 (0.13)d	7.38	7.57
ECS x T4	10	7.65 (0.08)e	7.59	7.71

Note: GPC, manual activation using gutta-percha cones; ECS, Easy Clean activation; T0, immediately after storage; T1, after 7 days; T2, after 14 days; T3 after 21 days; T4 after 35 days; SD, standard deviation; 95%CI, 95% confidence interval. Mean values followed by different letters indicate significant difference ($p < 0.05$).

Discussion

This study aimed to compare the different results for pH values of the deionized water, when GPC or ECS were used to agitate irrigants before $Ca(OH)_2$ paste filling. The null hypothesis tested was rejected because there was a significant difference between the techniques. From the 14th (T2) day on, ECS demonstrated a significant difference and higher mean pH values.

Conventional irrigation, such as manual techniques, have reduced ability to remove dentine debris, especially in anatomically complex areas[7,14]. The use of apically fitted GPC seems limited, since the volume of fresh solution in the apical region remains small, although some authors have revealed good results with this technique[24]. These characteristics may have influenced the better results achieved by ECS compared to GPC in this study. Some authors advocate the use of devices the use of irrigant activation techniques, allowing deeper areas of the dentine tubules to be reached[5,13], which is clinically relevant, resulting in a more favorable endodontic treatment[25].

It is known that the instrumentation of the root canal system, with manual or rotary instruments has its limitations, with the maintenance of untouched areas with microorganisms not reached and, thus, lead to endodontic treatment failure[8]. Thus, authors focused their investigations on irrigants with better cleaning and antibacterial activity, as a necessary complement to mechanical preparation[9]. NaOCl and EDTA are extensively studied irrigants, with physical actions that include smear layer and debris removal from the root canal walls[17,26], removal of root canal dressing[27], flow of irrigant into the lateral canals[28] and ability to penetrate dentinal tubules[29].

The ECS manufacturer suggests its use in reciprocation motion, but its use with continuous rotary movement at low speed has demonstrated more efficacy in cleaning the isthmus area as well as the root canal walls[18,26]. ECS presents a minimum risk of deforming the canal walls because it uses an acrylonitrile butadiene styrene (ABS) plastic instrument. It is therefore possible to introduce it up to the WL[18]. The technique is based on the premise that energy released by the instrument enhances the properties of the irrigants, promoting more effective debris and smear layer removal when compared with ultrasonic irrigation[17].

Regarding the use of NaOCl, beyond the physical aspects, the solution was chosen because of its recognized tissue-dissolving and antimicrobial capabilities[6]. At a concentration of 1% its effectiveness is recognized, which justifies having been elected in the present research[6]. Also, NaOCl seems to promote more uniform sealer penetration when compared to other irrigants[10]. On the other hand, EDTA solution, which has a basic pH, is also susceptible to agitation and this improves its results[30].

As the purpose of this study was to evaluate the agitation of the solutions prior to filling with $Ca(OH)_2$ paste, it is important to discuss this material. Its penetration in the dentinal tubules is essential for it to act[31] as an ally in combating microorganisms that remain in the root canal system even after cleaning and shaping.

The literature points out that the action of $Ca(OH)_2$ is associated with its pH and, depending on the vehicle used, this can occur faster (aqueous vehicle) or slower (viscous vehicle) due to ionic dissociation[21,22]. In this study it was opted for UltraCal paste (Utradent Products Inc., South Jordan, UT, USA), the vehicle of which is aqueous. Data highlight that the action of $Ca(OH)_2$ ranges from 7 to 45 days[32], but the greatest antimicrobial effect occurs in 14 days[33], which was corroborated by the results found here by analyzing the pH of the deionized water.

Considering the differences in the methods adopted, Plataniotis and Abbott[34] compared OH^- ion diffusion through root dentine taking various $Ca(OH)_2$ preparations, but they did not find significant differences. Eftekhar et al.[35] identified differences between three $Ca(OH)_2$ pastes regarding ion diffusion.

The study conducted by Batista et al.[23], using a similar method to that adopted here, revealed that high pH values were maintained, even after 30 days of filling with $Ca(OH)_2$ pastes manipulated with different vehicles: saline, propylene glycol, and *Aloe vera* gel.

Thus, it is evident that the results of this study depend on the technique used, and limitations may be related to this. The standardization of specimens by CBCT is a positive aspect. Likewise, the possibility of bias in the results was minimized by the fact that only one experienced operator conducted all the experiment.

Although this was an *ex vivo* study, the use of ECS for 30 seconds in continuous motion to agitate the NaCCl and EDTA solutions enhances the $Ca(OH)_2$ diffusion, providing higher pH values, from the 14th day on, when compared with manual agitation (GPC). Such results imply the recommendation of the technique that promotes better results, favoring the endodontic treatment with greater chances of success.

Acknowledgements

The authors declare that they have no conflicts of interests.

Data availability

Datasets related to this article will be available upon request from the corresponding author.

Conflicts of interest

None

Authors contribution

Conceptualization: Lucas Takeo Wakasugui, Liliane Roskamp, Natanael Henrique Ribeiro Mattos. Methodology: Camila Paiva Perin, Marilisa Carneiro Leão Gabardo, Flares Baratto-Filho, Natanael Henrique Ribeiro Mattos. Formal analysis and investigation: Lucas Takeo Wakasugui, Camila Paiva Perin, Allan Abuabara, Marilisa Carneiro Leão Gabardo. Writing - original draft preparation: Lucas Takeo Wakasugui, Camila Paiva Perin, Liliane Roskamp. Writing - review and editing: Allan Abuabara, Marilisa Carneiro Leão Gabardo, Flares Baratto-Filho. Resources: Lucas Takeo Wakasugui, Camila Paiva Perin, Natanael Henrique Ribeiro Mattos. Supervision: Flares Baratto-Filho, Liliane Roskamp, Natanael Henrique Ribeiro Mattos.

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