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Original Article

Effect of two methods of irrigant agitation on the temperature and cleanliness of sodium hypochlorite associated or not with a chelator

Mariana Maciel Batista Borges[1*], Mirela Cesar de Barros[1], Índia Olinta de Azevedo Queiroz[1], Flaviana Bombarda de Andrade[1], Marco Antonio Hungaro Duarte[1]

[1] Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo (FOB/USP), Bauru, Brazil.

Aim: This study investigated the influence *in vitro* of different sodium hypochlorite (NaOCl) agitation protocols associated or not with DualRinse (HEDP) on the temperature of the solution. **Methods:** Forty-eight premolars were instrumented and their apical third sealed to allow a closed irrigation system. The teeth remained immersed in a basin of warm water (37°C). The teeth were divided into the groups: G1 (NaOCl+Passive Ultrasonic Irrigation (PUI)), G2 (NaOCl/HEDP + PUI), G3 (NaOCl + EasyClean (EC)) and G4 (NaOCl/HEDP + EC). The canals were filled with the respective solutions and after 180 seconds the first temperature measurement was taken (T0). Then, the solutions were agitated, following the different protocols, for 60 seconds and a new measurement was performed (T60). The temperature was measured using a digital thermometer for type "K" sensors that was inserted into the middle third of the teeth. At the end of the measurements, the teeth were sectioned and prepared for scanning electron microscopy. The dentinal wall of middle third was graded according to the amount of debris and smear layer remaining on the walls. The results were analyzed using ANOVA test and Tukey's multiple comparisons ($p<0.05$). **Results:** G1 and G2 had an average increase in temperature of 1.1°C and 1.65°C, respectively ($p>0.05$). EasyClean caused a decrease in the temperature of the solutions in both groups, without a significant statistical difference with T0 ($p>0.05$). Regarding cleaning, it was only possible to observe clean dentinal tubules in the groups with the chelator. PUI discretely increased the temperature of the solution, regardless of the solution. The opposite effect was observed after activation with EasyClean. **Conclusion:** The association of NaOCl with a chelating agent promoted the cleaning of the dentinal tubules.

Keywords: Root canal irrigants. Sodium hypochlorite. Temperature. Ultrasonics.

Corresponding author:
Mariana Maciel Batista Borges
Department of Dentistry, Dental School of Bauru,
University of São Paulo - USP
Bauru, SP, Brazil
Postal Code 17012-901
Email address: mmbborges@usp.br
Tel: +55 14 32358000

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Introduction

Sodium hypochlorite (NaOCl) is the most used irrigant in endodontic treatment[1], due to its antimicrobial[2] and tissue dissolution[3] action, being used in concentrations between 0.5% - 6%[1]. In high concentrations, it may have a faster dissolution capacity[4,5], but its toxicity also increases in cases of extrusion[6], thus, methods to enhance the action of less concentrated solutions become relevant, such as agitation of the irrigant[5], constant refreshment and volume[7], and heating of the solution[8].

The increase in temperature of the NaOCl solution is related to an increase in reactivity and, therefore, a reduction in bacterial counts[9], potentiation of bacterial biofilm dissolution[10], and organic matter[5,8,11,12]. Although the dentin acts as a thermal insulator, the excessive increase in temperature could dissipate to the periapical tissues, causing damage to the bone tissue[13].

Among the solution heating methods, ultrasonic inserts seem to affect the temperature through the conversion of sound energy into heat[14], and, during cavitation, the insert can generate frictional heat against the canal walls[15,16]. The use of complementary cleaning methods, such as Passive Ultrasonic Irrigation (PUI), aims to improve disinfection by agitating the irrigant, pushing it to areas of anatomical complexities, since instrumentation and conventional irrigation fail to touch all walls[17]. PUI optimizes the removal of debris and bacteria[12], due to the shear force that generates tension in the canal wall and by the formation of bubbles that expand and collapse, producing a focus of energy[18].

Another alternative for agitation is the use of devices in continuous or reciprocal rotation, such as EasyClean (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil [US patent pending 61/849,608][17-20]. EasyClean is a plastic instrument, with a corresponding size of 25/0.04 and an "aircraft wing" shaped cross-section, composed of an acrylonitrile butadiene styrene. When used in continuous rotation, at low speed, it can produce a high swirl of the solution optimizing the cleaning of the dentinal walls[19,20]. Although the high speed can potentialize the effect in the solution, little is known about the device's influence on temperature.

Although NaOCl is the main solution of choice in endodontic treatment[1], it is unable to remove the inorganic components present in the root canal system[21], thus, its association with chelating solutions has been advocated[1]. Etidronate powder (Dual Rinse® HEDP Medcem Weinfelden, Switzerland) was proposed with the advantage of being added to NaOCl, reducing working time[22] and promoting continuous chelation[23], without affecting, in the short term, the properties of the NaOCl, such as antimicrobial action and tissue dissolution[22-29].

The use of the mixture of NaOCl with a chelator reduces the working time, since the removal of the smear layer occurs simultaneously with the removal of biofilm, as well as tissue dissolution, without the need for an additional clinical step, as with EDTA[22,24,28]. Experiments with the mixture demonstrated exposure of dentinal tubules after 5 minutes of irrigation, similar to the action of 17% EDTA[30], but without a substantial decrease in dentin microhardness, confirming that HEDP is a weak calcium-complexing that causes less change in dentin than other chelating agents such as EDTA or citric acid[30-32].

Chemical reactions occur more easily at high temperatures, mixtures of NaOCl and HEDP at body temperature showed a decrease in the amount of free available chlorine (FAC), reducing the therapeutic window of the solution[33], thus, it becomes important to determine if agitation methods are able to change the temperature of the mixture to predict the need for renewal and time of action of the solution. However, little is known about the influence of ultrasonic agitation and Easy Clean on the temperature change of this mixture into the root canal.

Therefore, the objective of this study was to investigate in vitro the impact of PUI and agitation with EasyClean on the temperature of the NaOCl solution associated or not with the chelating agent, etidronic acid (HEDP). Also, the cleanliness of dentinal tubules was evaluated using scanning electron microscopy (SEM).

The null hypothesis tested was that there is no difference in temperature after activation with the ultrasonic insertion and EasyClean (i) between the different irrigants tested (ii) and in the cleaning capacity of the dentinal tubules (iii).

Material and Methods

After approval by the ethics committee of the institution (approval No. 5.142.214), forty-eight single-rooted premolars extracted for periodontal and orthodontic reasons, donated with consent, were used. Teeth with fully formed apex with tooth lengths ranging from 20 to 22 mm, and with buccolingual diameter up to twice as wide as the mesiodistal diameter throughout the coronal two-thirds were selected. Canals with fin, lateral canal, apical curvature, isthmus, or two canals were excluded. A homogeneous distribution was made between the groups according to mesiodistal and buccolingual length after digital radiography and length.

The sample calculation was performed using G*Power v3.1 for Mac software (Heinrich Heine, Universität Düsseldorf). An alpha-type error of 0.05, beta power of 0.95, and a ratio of N2/N1 = 1 were stipulated. A total of 10 samples per group were indicated as a convenient size to observe significant differences. An additional 20% was calculated to compensate possible accidents during the procedures, thus 12 samples were used per group.

All teeth were prepared by a single operator. After coronal opening with 1014 diamond burs (KG Sorensen, Cotia, SP, Brazil) and removal of dentinal interferences with a 3082 bur (KG Sorensen, Cotia, SP, Brazil), the working length was determined after the introduction of a K#10 file (Dentsply, Maillefer, Switzerland) to its visualization in the greater foramen. From this length, 1 mm was subtracted.

The teeth were instrumented with a Sequence system (MK Life, Porto Alegre, RS, Brazil), using files: 15.04; 25.06; 35.04, and 40.04, with 400 RPM and 2N torque. At each file change, the root canals were irrigated with 5mL of 2% NaOCl (Rioquímica S/A, São José do Rio Preto, SP, Brazil), totaling 25 mL of irrigant at the end of the preparation.

The teeth were dried with a 40.04 absorbent paper cone (MK Life, Porto Alegre, RS, Brazil), and the apex was sealed with epoxy adhesive putty (Loctite Durepoxi, Henkel

LTDA, São Paulo, SP, Brazil) to allow a closed system and facilitate the final irrigation. To prevent the epoxy adhesive mass from entering the conduit, a main gutta-percha cone remained in the canal until the material was completely set.

The teeth were distributed in 4 groups (n=12) homogeneously based on the measurement of the entire length of the tooth to carry out the final irrigation protocol using different solutions:

G1- 2,5% NaOCl + ultrasonic agitation (PUI)

G2 – 2,5% NaOCL/HEDP (Dual Rinse®) + PUI

G3 - 2,5% NaOCl + activation with EasyClean (EC)

G4 - 2,5% NaOCL/HEDP + activation with EC

The sodium hypochlorite solution was used at an initial temperature of 23.3°C. In groups 2 and 4, the solution was prepared by mixing a capsule of Dualrinse® HEDP (4.5gr) with 50 mL of 2% NaOCl, according to the manufacturers' recommendations.

Initially, the teeth were filled with the solutions until it was possible to visualize the irrigant in the pulp chamber (±0.5mL). After 180 seconds, the first temperature measurement was taken (T0) and the solutions were agitated following the different protocols. In groups 1 and 3, Irrisonic ultrasonic insert (size 20, 0.01 taper; no cutting blades) (Helse, SR Viterbo, SP, Brazil) was used at low power (10%) for 60 seconds continuously. In groups 2 and 4, the irrigant was agitated with EasyClean, attached to the micromotor and contra-angle, at low speed with approximately 20,000 rotations per minute (KaVo Kerr Group, Charlotte, USA). The EasyClean tip was inserted 1mm short and Irrisonic 2 mm short of working length and agitated for 60 seconds.

During the entire experiment, the teeth remained fixed in a metallic structure to allow stabilization and were immersed in a water bath at a temperature of 37°C (Figure 1), with water up to the cervical region. In the end, for all groups, the highest temperature was recorded.

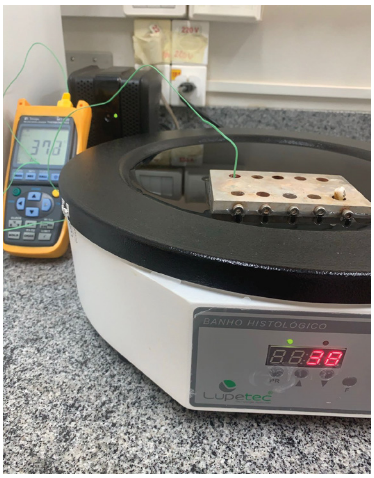

Figure 1. Tooth remained fixed in a metallic structure immersed in a water bath at a temperature of 37°C with water up to the cervical region.

The change in temperature was measured using a digital thermometer for type "K" sensors (Salvi Casagrande Ltda, São Paulo, SP, Brazil), coupled to a hypodermic probe with a length of 25 mm and a needle diameter of 0.2 mm type K (OMEGA Engineering inc.; Stamford, Connecticut, USA), with certified calibration (São Paulo Metrology Laboratory, Lamesp; Calibration Certificate No. CL -1981/2020). The probe was introduced 2 mm beyond the cervical region of the teeth.

Scanning electron microscopy (SEM) analysis

For SEM analysis, in each tooth, two opposite longitudinal grooves were made with diamond disks and, in the sequence, a vertical force was applied with a spatula to separate the halves. Mid-third images were obtained using SEM (Aspex Express; Fei Europe, Eindhoven, Netherlands), at an accelerating voltage of 15–20 kV and a standard magnification of ×500.

All images were saved in TIFF format and analyzed using Powerpoint software (Microsoft Corporation Redmond, WA, USA). The dentin surface was individually scored by two blind operators, based on Tartari et al., 2017[34]:

Score 1 - no smear layer, all dentinal tubules open;

Score 2 - small amount of smear layer, more than half of the dentinal tubules open;

Score 3 - homogenous smear layer covering the root canal wall, less than half of the dentinal tubules open;

Score 4 - complete root canal wall covered by a homogeneous smear layer, no open dentinal tubules.

Statistical analysis

A preliminary analysis of temperature data normality was obtained using the Shapiro-Wilk test and showed normal distribution. ANOVA test and Tukey's multiple comparisons test ($p<0.05$) were used to compare the temperature between groups and Paired T-Test was used for intragroup comparisons. In the analysis of the cleaning of the dentinal tubules, the Kappa test was applied to determine the agreement between the examiners. The non-parametric data of cleanliness were compared using Kruskal-Wallis and Dunn's multiple comparisons test ($p<0.05$).

Results

Table 1 shows the mean and standard deviation of the initial temperature values (T0) and after the agitation protocols with the different solutions. After PUI for 60 seconds continuously, there was a statistically significant difference in the intracanal temperature of the two tested solutions ($P<0.05$). EasyClean, in both groups, generated a decrease in the temperature of the solutions, with no statistical difference between T0 and T60.

Table 1. Mean and standard deviation of the initial temperature and after 60 seconds of agitation and the difference between them.

Groups	IT (°C) Mean ± SD	FT (°C) Mean ± SD	IT-FT
NaOCl + PUI	32,2 ± 3,093^{A}	33,57 ± 2,84^{B}	1,1°C^{ab}
NaOCl/HEDP + PUI	32,36 ± 1,56^{A}	34,17 ± 1,58^{B}	1,65^{b}
NaOCl + EC	30,4 ± 2,5^{A}	29,97 ± 2,18^{A}	-0,3^{a}
NaOCl/HEDP + EC	29,2 ± 2,25^{A}	29,2 ± 1,75^{A}	-1^{a}

IT, initial temperature; FT, final temperature.
SD, standard deviation.
Different capital letters represent statistical significant difference between columns. Different lower case letters represent statistical difference between lines (p>0.05).

Regarding the temperature difference, the ultrasonic activation of sodium hypochlorite + Dualrinse was higher (1.65°C) and statistically different (P<0.05) when compared to the groups in which the EasyClean was used.

As for wall cleaning (Table 2), the inter-examiner agreement was high (kappa = 0.90). Regardless of the agitation method, the dentinal tubules without smear layer were only observed in the groups in which the sodium hypochlorite solution was associated with HEDP (G2 and G4) (Figure 2).

Table 2. Median and minimum and maximum values of the scores of dentin surface after analysis using scanning electron microscopy.

Groups	Cleaning
G1 - NaOCl + PUI	4^{A} (3-4)
G2 - NaOCl/HEDP + PUI	2,5^{B} (1-4)
G3 - NaOCl + EC	4^{AB} (1-4)
G4 - NaOCl/HEDP + EC	3^{B} (3-4)

Different capital letters represent statistical difference between groups (p>0.05).

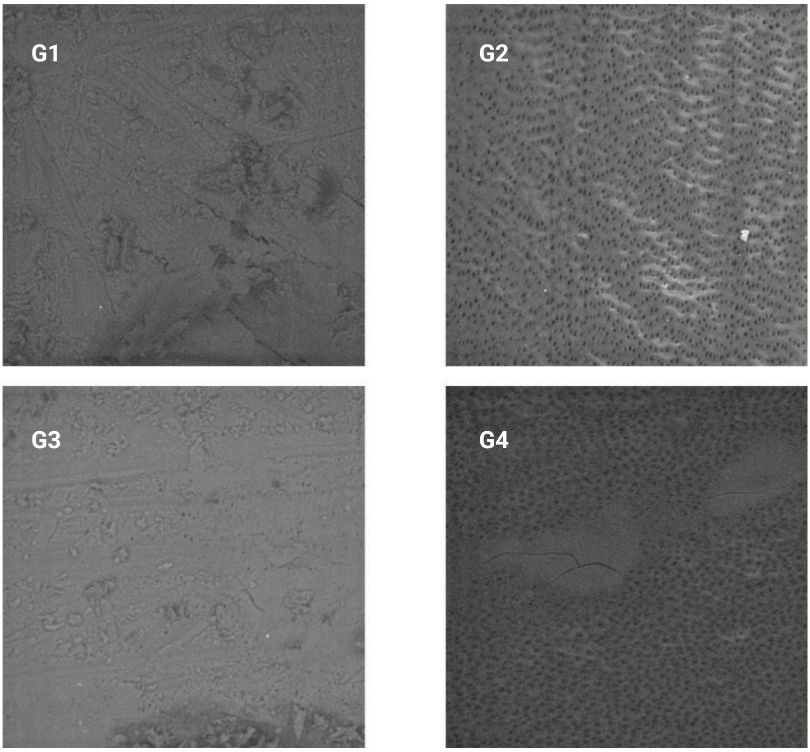

Figure 2. Scanning electron microscopy images of the dentin walls after irrigation protocols (G1- 2,5% NaOCl + ultrasonic agitation (PUI), G2 – 2,5% NaOCL/HEDP (Dual Rinse®) + PUI, G3 - 2,5% NaOCl + activation with EasyClean (EC), G4 - 2,5% NaOCL/HEDP + activation with EC)

Discussion

The effect of different agitation protocols on the intracanal temperature of different irrigants was evaluated. The ultrasonic insertion for 60 seconds continuously was able to increase the temperature of all the solutions tested, however, agitation with EasyClean had the opposite effect. The cleaning of the dentinal tubules was directly dependent on the chemical action of the solution. Thus, the null hypothesis tested there is no difference in temperature after activation with the ultrasonic insertion and EasyClean was rejected, however the null hypothesis that there is no difference between the solution was accepted. The null hypothesis that there is no difference in the cleaning capacity of the dentinal tubules was also rejected.

The chemical efficiency of NaOCl (reaction rate), as well as its effectiveness (tissue dissolution), are directly influenced by concentration, exposure time, ultrasonic activation, contact area, and temperature[35]. Heating is a simple procedure that can positively impact disinfection[9] and tissue dissolution[8], in this sense, quantifying the temperature is important to avoid damage to periapical tissues[13], determining the amount of FAC, in the case of mixtures such as NaOCl + HEDP, since the intracanal temperature can affect the behavior of instruments manufactured with martensite phase, used during preparation[36].

HEDP is an alkaline chelator that works in the pH range of 10.8 -12.3 and has been mixed with NaOCl for continuous irrigation and chelation with the aim to simplify the clinical technique and improve the removal of smear layer from the root canal[37]. Álvarez-Sagues et al.[38], 2021, demonstrated that HEDP mixed with NaOCl was the most effective irrigant in the removal of mature biofilm, since no bacteria were recovered after treatment of this group, followed by EDTA mixed with NaOCl and, finally, the EDTA-irrigated group. Chelators are related to the promotion of the detachment of biofilm from the root canal walls (Wright et al.[33], 2019) once the solution can bind metal ions required for bacterial metabolism[4].

Despite the advantages, in continuous chelation, the constant reaction with NaOCl and the chelator can promote the reduction of FAC and with that decrease the antimicrobial action and tissue dissolution[8]. Heating the solution promotes an acceleration of this chemical reaction between the irrigants. Tartari et al.[8] (2021) demonstrated that heating the NaOCl+HEDP mixture increased organic dissolution and smear layer removal, however, it needed more renewal to retain its effects when heated, due to chlorine depletion. Wright et al.[33] (2019) observed that heating the mixture, NaOCl+HEDP, at 35ºC reduced the therapeutic window of the solution to 20 minutes.

In our study, the only agitation method that promoted heating was PUI, corroborating other investigations[14,39,40].The findings of the present study demonstrated a maximum heating of 34.17°C obtained after PUI in the NaOCl+HEDP group. Although it is below 35°C, it is prudent to renew the mixture among activation cycles and after to maintain the properties of the solution.

On the other hand, the Easy Clean file did not influence the intracanal temperature. Although the flexible tip works at a high speed (20,000 RPM), the frictional forces between the solution and the root canal walls were not able to generate heat, unlike the action mechanism of the ultrasonic insert[36]. Probably, once it is manufactured by a polymer, Easy Clean does not favor heat production as a metallic insert. Reviewing the literature, there are no publications that allow comparisons with the results obtained using this instrument. Zeltner et al.[15] (2009) analyzed the influence on the ultrasonic activation temperature with files of different calibers (#15, #25, #35), as well as with thin ultrasonic insert, and observed that the most robust file (#35) was able to promote the maximum increase in temperature, confirming the influence of the metal, as well as its diameter, in the conduction of intracanal heat. Such a finding may help to understand the performance of the Easy clean file.

In our study, the teeth remained in a water bath, simulating the clinical situation, since previous studies demonstrated rapid cooling of the solution before the end of irrigation[14]. Thus, the initial temperature recorded was much higher than the value of the solution at room temperature, due to heat transfer from the environment to the solution. To measure the temperature, the positioning of the probe was standardized in the middle third of the teeth (2 mm below the dentin-enamel limit), due to the ease of attaching the probe, and for being the region with the largest reservoir of the solution. Considering the proposed recommended ultrasonic activation protocol of 3 cycles of 20 seconds[18], the results obtained showed that the limited time of agitation was not enough to significantly heat the solution. In addition, dentin exerts a buffering effect, promoting thermal dissipation of heat[41].

Leonardi et al.[14] (2019) demonstrated that the heating obtained after ultrasonic activation was dependent on time, as well as on the third of the canal, with 60 seconds showing higher values. In the apical third, the mean temperature value was higher. the average time for the temperature to return to 37ºC was 43 seconds, showing that the heated irrigant was limited to the time during it remained warm in the canal.

Other studies have also demonstrated limited heating of the solution after ultrasonic activation[41]. With the constant renewal and high flow of the irrigant, it seems to be impossible for the solution to reach body temperature. In our study, activation was performed only after 180 seconds of immersion of the solution in the root canal, and an initial heating was observed due to the absorption of heat from the environment. The irrigating solution acts through convection and diffusion, the latter being the phase in which the solution remains at rest. The random movement of the liquid particles favors the interaction with the substrate[42]. Considering the findings of this study, diffusion can also help to obtain heat from the liquid, prior to the ultrasonic activation.

During the chemical-mechanical preparation, a thin layer of organic and inorganic matter, called smear layer, is formed[21]. The removal of this residual layer is important to the success of the treatment because it is related to the penetrability of antimicrobial substances in the dentinal tubules[43]. In our study, at the end of the irrigation protocols, the dentinal walls were evaluated under a scanning electron microscope, as it is an important tool that allows the observation of the dentin surface in detail[34]. The findings of our study showed that the removal of inorganic matter in tubules is primarily achieved by the chemical action of the substance since even with the agitation of the sodium hypochlorite solutions for 60 seconds, the solution itself was not able to remove the smear layer, corroborating the literature[1,34]. Therefore, a chelating agent is always recommended for the removal of inorganic matter.

Considering the conditions of the present study, the ultrasonic activation discretely increased the temperature of NaOCl solution, associated or not with HEDP. On the other hand, the activation of the solutions with Easy Clean did not generate heating. The smear layer removal is directly related to the chemical action of the chelator, independently of the kind of the agitation device.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Data Availability

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

Author Contribution

- Mariana Maciel Batista Borges and Marco Antonio Hungaro Duarte: Methodology, validation, investigation, data curation, writing – original draft preparation and resources

- Mirela Cesar de Barros: Methodology and execution

- Índia Olinta de Azevedo Queiroz: Conceptualization and methodology

- Flaviana Bombarda de Andrade: Validation and writing – review, and editing.

All authors actively participated in the manuscript's findings and revised and approved the final version of the manuscript.

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