Effect of the Controlled Delivery of Chelating Agents on the Pulp Tissue Dissolution Ability of Fresh Sodium Hypochlorite Solutions

Natalia Navarrete, DDS, PhD,[*] Juan M. Almansa, MSc,[†] José C. de la Macorra, DDS, MS, PhD,[†] María Teresa Arias-Moliz, DDS, PhD,[‡] and Ana Arias, DDS, MS, PhD[†]

ABSTRACT

Introduction: The purpose of this study was to evaluate pulp tissue dissolution ability of sodium hypochlorite (NaOCl) when mixed with tetrasodic etidronate (Na_4HEBP) and disodic ethylenediaminetetraacetate (Na_2EDTA) under controlled flow. **Methods:** Bovine pulp tissue was extracted from the lower incisors of 10 bovine jaws. Pulp specimens were standardized in size (1 × 3 × 10 mm), blotted dry, and weighed (initial weight [T0]: mean (SD) = 31.98 (1.18) mg). Specimens from the same jaw were randomly assigned to 2 control (n = 3 each) and 4 experimental groups (n = 10 each): NC (negative control/distilled water), PC (positive control/6%NaOCl), G1 (3%NaOCl), G2 (3%NaOCl-17% Na_2EDTA), G3 (3%NaOCl-18% Na_4HEBP), and G4 (3%NaOCl-9% Na_4HEBP). Distilled water and NaOCl were provided using a delivery pump under a continuous controlled rate (1 mL/min). A second pump alternately delivered either Na_2EDTA or Na_4HEBP at the same rate with a 30-second programmed interval. Percentage of tissue weight loss was calculated at 2, 5, and 10 minutes (T2, T5, and T10) and compared among groups with analysis of variance. Free available chlorine and pH were controlled at T0 and T10. **Results:** No tissue remained in PC at T5. No dissolution occurred in NC. There were no significant differences in the percentage of weight loss among experimental groups at any point of time. Some remnant tissue was found in G3 (1.4% ± 2.4) and G4 (1.6% ± 2.3) at T10, whereas nothing was left in G1 and G2. **Conclusions:** The controlled delivery of Na_2EDTA and Na_4HEBP did not alter tissue dissolution ability of NaOCl when fresh solutions were mixed in the root canal. *(J Endod 2023;49:307–312.)*

SIGNIFICANCE

Contrary to the findings from studies that immersed tissue in premixed solutions of NaOCl and chelating agents, the controlled delivery of EDTA and etidronate did not alter tissue dissolution ability of NaOCl when fresh solutions were mixed in the root canal.

KEY WORDS

Tissue dissolution; controlled delivery; sodium hypochlorite; NaOCl; EDTA; etidronate; HEBP; HEDP

The combination of mechanical preparation and chemical disinfection is necessary for a successful endodontic therapy[1,2]. Mechanical instrumentation alone cannot reach the internal anatomic complexities of the root canal system[3]. For this reason, manual or rotary root canal preparation needs to be combined with irrigating solutions. Since the ideal irrigant solution has not been described yet, a combination of irrigants is needed for proper tissue dissolution, reduction of biofilm, cleaning of unprepared areas and removal of debris, and the smear layer produced on the dentinal tubules by the action of endodontic shaping instruments[4,5].

Sodium hypochlorite (NaOCl) is the most effective irrigating solution due to its organic tissue dissolution capacity and elimination of bacteria biofilm; however, it is not able to eliminate the inorganic matter[1]. It is a strong oxidizing agent with an alkaline pH that relieves free available chlorine in solution. NaOCl is used in endodontics at varying concentrations from 1%–6%[1]. The properties of NaOCl depend on its concentration, temperature, pH, frequency and intensity of mechanical agitation, and solution refreshment. Importantly, it is also affected by the surface area of the exposed tissue[6-9].

Chelating agents, such as different salts of ethylendiaminetetraacetic acid (EDTA), are used to remove the inorganic matter exerting a minimal tissue dissolution capacity and antimicrobial activity[10,11]. EDTA is a polyprotic acid, normally used in endodontics at concentrations varying from 10%–17%.

From the *Department of Clinical Dentistry, Universidad Europea de Madrid, Faculty of Biomedical and Health Sciences; †Department of Conservative and Prosthetic Dentistry, School of Dentistry, Complutense University, Madrid; ‡Department of Microbiology, School of Dentistry, University of Granada, Granada, Spain

Address requests for reprints to Dr Ana Arias, Department of Conservative and Prosthetic Dentistry, School of Dentistry, Complutense University, Plaza Ramon y Cajal s/n, Ciudad Universitaria, 28040 Madrid, Spain.
E-mail address: aariaspa@ucm.es
0099-2399

https://doi.org/10.1016/j.joen.2022.12.009

Different forms of the salt of EDTA have been described: disodium (Na_2EDTA), trisodium (Na_3EDTA), and tetrasodium EDTA (Na_4EDTA)[12].

Etidronate (also known as 1-hydroxyethylidene-1, 1-bisphosphonate or Na_4HEBP) is a weak and biocompatible chelator with a pH varying from 10–12[13]. Its use in combination with NaOCl has been suggested to reduce the formation of smear layer and debris accumulation during mechanical preparation of the root canal; however, it requires longer contact times to achieve a similar chelating effect as EDTA (5 minutes vs 1 minute)[14,15].

At the same time, chelators, like EDTA, interacted with NaOCl affecting drastically its tissue dissolution ability by reducing active chlorine content[16-21]. For this reason, the mixture and alternate use of NaOCl and EDTA during root canal preparation was discouraged, and the use of chelators was only recommended at the end of the shaping procedure instead[18]. This effect was not observed with Na_4EDTA or HEBP that, when combined with NaOCl, the solution promoted smear layer removal and organic matter dissolution[22,23]. Moreover, NaOCl did not affect the calcium chelation ability either of EDTA or HEBP[19,23].

Nevertheless, the most published research on the dissolution effect of root canal irrigants has been carried out in static models with mixed solutions at different ratios[9,17,18,20-22,24] in which the active chlorine content and tissue dissolution properties were altered when submerging different tissue specimens in previously mixed solutions. The specimens used in the different studies varied from porcine[18,25,26], to bovine[8,21,24] and human[9,27] tissues, as well as rats[28] and rabbits[6]. However, these models differ from the clinical situation in which NaOCl solution is delivered under a controlled flow and is continuously refreshed during the shaping procedure. It is, therefore, relevant to analyze the interaction between the irrigation solutions and the organic matter under a more realistic model that reproduces the delivery and refreshment of irrigants. The purpose of this study was to evaluate pulp tissue dissolution ability of refreshed NaOCl when mixed with Na_4HEBP (both at 9% and 18%) and Na_2EDTA (17%) under a controlled flow.

MATERIALS AND METHODS

Pulp Specimen Preparation

Bovine pulp tissue was extracted from the 6 mandibular incisors of 10 bovine jaws (30–70 months old) dissected after being slaughtered for food production. A total of sixty intact fresh bovine anterior mandibular teeth were extracted within 48 hours after slaughtering and stored in glass vials with 0.1% thymol solution. Crowns were removed at the cementum–enamel junction and roots sectioned at 3 mm from the apex with a microtome (0.2 mm/D64, Leica Microsystem, Barcelona, Spain). Pulp tissue was then carefully removed with a periodontal probe and cotton pliers and rinsed with distilled water to remove debris and excess of blood (Fig. 1).

All samples were individually stored in 1.5 mL Eppendorf tubes (Deltalab, S.L., Barcelona, Spain) with 1 mL distilled water at -20°C until further use. Before the test, the tubes with the specimens were maintained at room temperature for 30 minutes and later placed in a hot water bath at 37°C (Precisterm Selecta, Barcelona, Spain) for 15 minutes to simulate clinical conditions.

Pulp specimens were then standardized in size (1 × 3 × 10 mm) under magnification (loupes 3.8, ExamVision Akura Medical, NovaMed Concepts S.L., Madrid, Spain) using a millimeter graph paper vector sheet, a stainless-steel surgical scalpel blade nº15 (Braun, Tuttlingen, Germany), and a digital gauge (Mitutoyo, Guipuzcoa, Spain) (Fig. 1). Specimens were blotted dry for 30 seconds and weighed using a hermetic precision electronic balance (Sartorius AG, Göttingen, Germany) before the test to determine baseline weight (T0) of the samples.

Groups and Solutions

The pulp specimens obtained from the 6 anterior teeth of the same bovine jaw were randomly distributed into 4 experimental groups ($n = 10$) and 2 control groups ($n = 3$) according to the irrigation protocols as follows:

G1—Continuous delivery of 3% NaOCl (CanalPro 3% NaOCl, Coltène/Whaledent, GmbH & Co KG, Langenau, Germany).

G2—Continuous delivery of 3% NaOCl + alternate delivery of 17% Na_2EDTA (CanalPro EDTA 17%, Coltène/Whaledent).

G3—Continuous delivery of 3% NaOCl + alternate delivery of 18% Na_4HEBP (Na_4HEBP, Cublen K8514 GR; Zschimmer & Schwarz, Moshsdorf GmbH & Co KG, Burgstädt, Germany).

G4—Continuous delivery of 3% NaOCl + alternate delivery of 9% Na_4HEBP (Na_4HEBP, Cublen K8514 GR; Zschimmer & Schwarz).

NC (negative control)–Distilled water.

PC (positive control)–Continuous delivery of 6% NaOCl (CanalPro 6% NaOCl, Coltène/Whaledent).

HEBP solutions were freshly prepared before the tests. The white granules of HEBP tetrasodium salt (85% active salt) were dissolved in distilled water to obtain 18% and 9% HEBP solutions.

Tissue Disolution Method

Each sample was introduced into a centrifuge tube that contained an inner filter (Ultrafree-MC-HV, Merck Millipore, Germany). The filter was placed in a 50 mL plastic tube (Cellstar graduated polypropylene tubes, Greiner Bio-one8, VWR, Pennsylvania, USA) to collect the supernatant.

Both distilled water (in the NC group) and fresh NaOCl (in PC group and experimental groups) were continuously delivered with a pump (CADD-Prizm VIP, model 6101, Smiths Medical ASD, Inc, Minnesota, USA) and a 24G/9 mm needle under a continuous controlled rate (1 mL/min). A second pump and a second needle alternately delivered either EDTA (in G2) or HEBP (18% Na_4HEBP in G3 and 9% Na_4HEBP in G4) at the same rate with a 30-second programmed interval. The delivery of NaOCl with the first pump was not interrupted when the second pump was activated. Both needles were fixed to the cap of the plastic tube, allowing the individual solutions not to contact before the test (Fig. 1).

The pumps were stopped after 2 minutes of application of the irrigant solutions. Samples were then washed with 2 mL of distilled water for 30 seconds to neutralize the effects of the different solutions, blotted dry again and reweighed to evaluate remnant tissue (T2). The same procedure was repeated after 5 (T5) and 10 (T10) minutes.

Available Chlorine, pH and Temperature

Three samples in each group were randomly selected for further registration of free available chlorine and pH at both T0 and T10. A standard iodine/thiosulfate titration method[29] was used to calculate available chlorine content in the NaOCl solutions at T0 and T10 in all study groups. Initial and final pHs of each solution were registered with a calibrated pH-meter (MicropH 2001, CRISON, Hach Lange, Spain, S.L.U.). Final temperature of supernatant solutions was also registered.

Statistical Analysis

Percentage of remnant tissue was calculated at T2, T5, and T10 using the formula: 100-([(initial weight-final weight)/initial weight] *100).

After confirming the assumption of normal distribution of data, a factorial repeated measures analysis of variance (ANOVA) was used to detect any overall difference in the percentage of remnant tissue across time

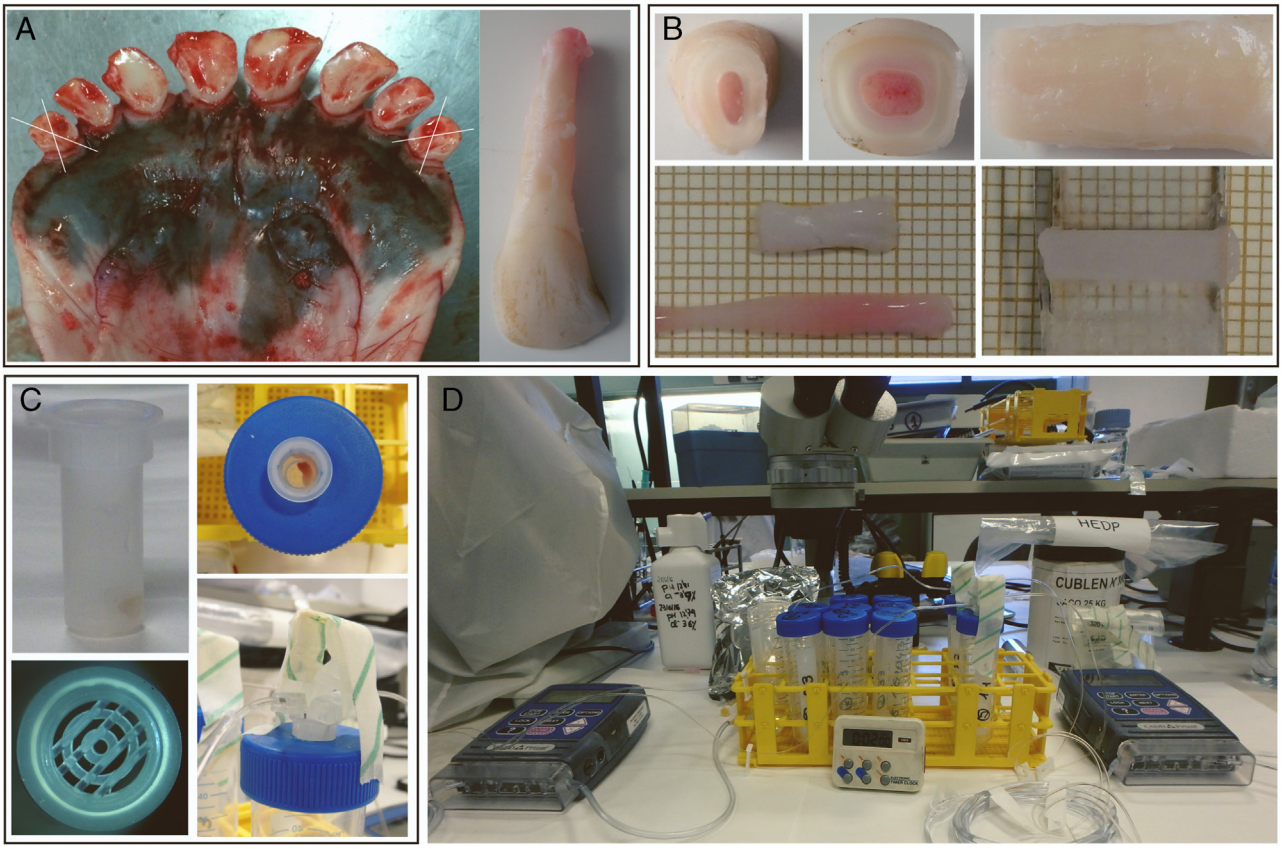

FIGURE 1 – A schematic representation of specimen preparation and arrangement of needles and pumps: (*A*) Bovine jaw and incisor; (*B*) root sectioning, pulp tissue removal, and standardization; (*C*) introduction of tissue sample in centrifuge tube with inner filter to collect the supernatant and needles fixation; (*D*) delivery pumps attached.

points and groups. A Greenhouse–Geisser correction was applied to the repeated-measure ANOVA because the data violated the assumption of sphericity. Tukey post hoc test was used for pairwise comparisons when repeated measures ANOVA rendered significant differences. Descriptive statistics was used for the rest of the variables: available chlorine, pH, and temperature of the different solutions.

RESULTS

The mean percentages and standard deviations of remnant tissue over time for each group are shown in Table 1. The mean initial weight (T0) of the pulp specimens was 31.98 mg (±1.18).

No tissue was remnant in PC at T5. Tissue dissolution was not observed in NC.

There was a significant reduction in the percentage of remnant tissue over time in all groups ($P < .001$). No significant differences in the percentage of weight loss among experimental groups were obtained at any point time, except for G4 that showed significant more remnant tissue at both T2 and T5 ($P < .001$) than G1, although no significant differences were detected when compared to G2 and G3. At the same time there were no significant differences among groups at T10; however, some remnant tissue was found in G3 (1.4% ± 2.4) and G4 (1.6% ± 2.3) at T10, whereas nothing was left in G1 and G2 (Table 1).

Values of available chlorine and pH for each group at T0 and T10 are shown in Table 2. Available chlorine did not change in the supernatant for both PC and G1; however, a reduction in the available chlorine was observed after 10 minutes (T10) in the rest of the groups.

Other reactions were also observed in the supernatant of G2: a decrease in the initial pH, the presence of bubbles due to gas formation, and an increase in the temperature (35°C). This exothermic reaction was not observed in the rest of the groups (Table 2). Moreover, at the end of the experiment the filter containing the pulp specimen presented salt precipitate in G2.

DISCUSSION

The present study assessed the interaction of NaOCl with 17% Na_2EDTA, and 9% and 18% Na_4HEBP under a controlled flow. The dynamic model developed allowed the refreshment of irrigation solutions to simulate the delivery that occur in real conditions; and hence allowed to determine tissue dissolution ability of irrigation solutions from a realistic clinical approach.

In the current study, bovine pulp tissue was used due to the similarity to human pulp[30], it is readily available and can be cut into standardized specimens in terms of size and homogeneity among groups. The age of the animals varied from 30–70 months to obtain an adequate and standardizable pulp surface area. Thus, samples were standardized in size and presented similar weights. Moreover, in order to reduce the individual variability among groups, 6 mandibular incisors of the same bovine jaw were randomly distributed to the 4 experimental and 2 control groups, so that there were pulp tissue samples of each bovine animal in all the groups. This strategy reduced the chances of individual confounding factors affecting the outcome of the study. To the authors' knowledge, no study has so far analyzed the effect of HEBP on the dissolution capacity of NaOCl in bovine pulp tissue, although Tartari et al[24] and Ulusoy et al[31]

TABLE 1 - Mean Percentages and SD of Remnant Tissue in the Different Groups Over Time

	Remnant tissue (%)			
	Initial (T0)	2 min (T2)	5 min (T5)	10 min (T10)
NC: Distilled water	100	98.3 ± 0.4	111.1 ± 5.1	123.1 ± 6.3
PC: 6% NaOCl	100	13.2 ± 2.3	0	0
G1: 3% NaOCl	100	49.6 ± 14.3	12.24 ± 9.3	0
G2: 3% NaOCl-17% Na_2EDTA	100	52.3 ± 7.8	19.4 ± 6.8	0
G3: 3% NaOCl-18% Na_4HEBP	100	58 ± 10.7	18.5 ± 9.8	1.4 ± 2.4
G4: 3% NaOCl-9% Na_4HEBP	100	62.3 ± 7.9	23.2 ± 8.1	1.6 ± 2.3

NC, negative control; PC, positive control.

analyzed the dissolution of bovine muscle tissue submerged in previously mixed irrigation solutions.

The dissolution capacity of irrigation solutions has been evaluated with different models. Indirect methods have been used to test the changes produced in irrigation solutions; for example, determination of the presence of hydroxyproline or phosphate[32,33] and the amount of available chlorine[23]. However, the solutions may disturb the determinations in some biochemical assays. Since tissue dissolution capacity is related to OCl^{-}[6], the amount of available chlorine was tested in the current study in initial and final solutions through iodometric titration. Other studies used direct methods, for example the histological evaluation of the percentage of remaining pulp tissue in root cross-section of vital extracted teeth[27], the time needed for partial or total tissue dissolution[9,21,28], and loss of tissue weight by immersing the sample in static solutions or in artificial grooves[24,26,31]. The current study tested the percentage of remnant tissue over time but using a pump that allowed the refreshment of irrigation solutions and a controlled delivery of irrigants at a standardized flow rate (0.017 mL sec^{-1}). While the optimal volume of irrigant has not been determined yet, the efficacy of irrigation varied with different operators[34]; hence, it is important to standardize the flow rate in this type of studies.

Like in previous studies[8,26], no pulp tissue dissolution was observed when distilled water was used and an increase in the percentage of remnant tissue occurred after 5 and 10 minutes due to tissue hydration. However, results were different for PC group in comparison with static studies[9,21], complete pulp dissolution was observed after a 5-minute delivery of 6% NaOCl. The use of a continuous flow that allowed the refreshment of NaOCl solution reduced the time needed to half and a complete dissolution of the sample was obtained in comparison with previous studies[9,21].

No significant differences in the percentage of remnant tissue among experimental groups were observed at any point of time. Again, these results differed from those obtained in previous studies that immersed tissue in premixed solutions. Tartari et al[24] observed that HEBP minimally affected tissue dissolution ability of NaOCl compared to EDTA. Furthermore, EDTA inactivated NaOCl and no tissue dissolution occurred according to previous studies[8,18,20,21]. When NaOCl and EDTA solutions are mixed, a reaction occurs that neutralizes NaOCl, decreasing available chlorine (OCl^{-}). This decrease was also observed in the present study when the recollected supernatant NaOCl-EDTA was analyzed. This decrease was expected since the test model used in the present study allowed the continuous refreshment of the solutions in contact with the sample tissue; however, the supernatant was collected in a closed system. The model combined a centrifuge tube with an inner filter for the pulp tissue sample and a 50 mL plastic tube, where the centrifuge tube was placed for supernatant collection. For this reason, when NaOCl and EDTA are not premixed, but simultaneously delivered and refreshed, it seemed not to meaningfully alter the available chlorine, although a reduction occurred in the collected supernatant. An exothermic reaction, the reduction in the pH and the presence of bubbles[19,21,23] were also observed in the supernatant in group 2. Previous studies have also reported the increase in temperature of the mixed NaOCl-EDTA solutions at high concentrations (5%) of NaOCl[17,20,23]. If EDTA and NaOCl solutions would have been mixed before the test, instead of being delivered separately like in the present study, tissue dissolution could have been affected like in previous studies[18,20,21]. At the same time, free available chlorine has been shown to suffer a rapid consumption when in contact with dentin, decreasing the ability to dissolve the organic tissue[35]. Although this has been demonstrated in previously mixed irrigation solutions without NaOCl refreshment, the absence of dentin in the present study could lead to an over estimation of the dissolution activity of the solutions. The current study demonstrated that the alternate delivery of EDTA and HEBP under controlled flow did not affect the pulp tissue dissolution ability of refreshed NaOCl. Further research should confirm its effect in the presence of dentin and preferably in human root canals[36].

With the limitations of this *in vitro* study, it can be concluded that the controlled delivery of EDTA and HEBP did not alter tissue dissolution ability of NaOCl when fresh solutions were mixed in the root canal.

TABLE 2 - Available Chlorine and pH of the Different Solutions at T0 and T10, and the Mean Final Temperature

		Available chlorine			pH		Temperature (°C)
Group	Components	Initial	Final	% remnant	Initial	Final	Final
PC	6% NaOCl	6.4	6.4	100	12.89	12.92	25
G1	3% NaOCl	3.9	3.8	97.4	12.81	12.77	25.33
G2	3% NaOCl	3.9	—	—	12.81	—	—
	17% Na_2EDTA	—	—	—	8.57	—	—
	NaOCl + EDTA	—	1.7	43.6	—	9.47	35
G3	3% NaOCl	3.6	—	—	12.81	—	—
	18% Na_4HEBP	—	—	—	10.93	—	—
	NaOCl + HEBP	—	2.4	66.7	—	12.70	26.67
G4	3% NaOCl	3.6	—	—	12.81	—	—
	9% $HEBPNa_4$	—	—	—	11.13	—	—
	NaOCl + HEBP	—	2.2	61.1	—	12.63	27.33

PC, positive control.

ACKNOWLEDGMENTS

The authors deny any conflicts of interest related to this study.

The authors thank to all the staff in the research laboratory of the School of Dentistry at Complutense University.

REFERENCES

1. Zehnder M. Root canal irrigants. J Endod 2006;32:389–98.
2. Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP. The effectiveness of different acid irrigating solutions in root canal cleaning after hand and rotary instrumentation. J Endod 2006;32:993–7.
3. Peters OA, Laib A, Göhring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod 2001;27:1–6.
4. Paqué F, Laib A, Gautschi H, Zehnder M. Hard-tissue debris accumulation analysis by high-resolution computed tomography scans. J Endod 2009;35:1044–7.
5. Zhao D, Shen Y, Peng B, Haapasalo M. Root canal preparation of mandibular molars with 3 nickel-titanium rotary instruments: a micro-computed tomographic study. J Endod 2014;40:1860–4.
6. Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. Int Endod J 1982;15:187–96.
7. Rossi-Fedele G, Guastalli AR, Doğramaci EJ, et al. Influence of pH changes on chlorine-containing endodontic irrigating solutions. Int Endod J 2011;44:792–9.
8. Stojicic S, Zivkovic S, Qian W, et al. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod 2010;36:1558–62.
9. Cullen JKT, Wealleans JA, Kirkpatrick TC, Yaccino JM. The effect of 8.25% sodium hypochlorite on dental pulp dissolution and dentin flexural strength and modulus. J Endod 2015;41:920–4.
10. Hülsmann M, Heckendorff M, Lennon Á. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J 2003;36:810–30.
11. Ballal NV, Mala K, Bhat KS. Effect of maleic acid and ethylenediaminetetraacetic acid on the dissolution of human pulp tissue–an *in vitro* study. Int Endod J 2011;44:353–6.
12. Tartari T, Bachmann L, Zancan RF, et al. Analysis of the effects of several decalcifying agents alone and in combination with sodium hypochlorite on the chemical composition of dentine. Int Endod J 2018;51:e42–54.
13. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod 2005;31:817–20.
14. De-Deus G, Zehnder M, Reis C, et al. Longitudinal co-site optical microscopy study on the chelating ability of etidronate and EDTA using a comparative single-tooth model. J Endod 2008;34:71–5.
15. Paqué F, Rechenberg DK, Zehnder M. Reduction of hard-tissue debris accumulation during rotary root canal instrumentation by etidronic acid in a sodium hypochlorite irrigant. J Endod 2012;38:692–5.
16. Rossi-Fedele G, Doramac EJ, Guastalli AR, et al. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012;38:426–31.
17. Prado M, Santos Júnior HM, Rezende CM, et al. Interactions between irrigants commonly used in endodontic practice: a chemical analysis. J Endod 2013;39:505–10.
18. Grawehr M, Sener B, Waltimo T, Zehnder M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J 2003;36:411–5.
19. Grande NM, Plotino G, Falanga A, et al. Interaction between EDTA and sodium hypochlorite: a nuclear magnetic resonance analysis. J Endod 2006;32:460–4.
20. Clarkson RM, Podlich HM, Moule AJ. Influence of ethylenediaminetetraacetic acid on the active chlorine content of sodium hypochlorite solutions when mixed in various proportions. J Endod 2011;37:538–43.
21. Irala LED, Grazziotin-Soares R, Salles AA, et al. Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA. Braz Oral Res 2010;24:271–6.
22. Tartari T, Oda DF, Zancan RF, et al. Mixture of alkaline tetrasodium EDTA with sodium hypochlorite promotes *in vitro* smear layer removal and organic matter dissolution during biomechanical preparation. Int Endod J 2017;50:106–14.
23. Biel P, Mohn D, Attin T, Zehnder M. Interactions between the tetrasodium salts of EDTA and 1-hydroxyethane 1, 1-diphosphonic acid with sodium hypochlorite irrigants. J Endod 2017;43:657–61.

24. Tartari T, Guimarães BM, Amoras LS, et al. Etidronate causes minimal changes in the ability of sodium hypochlorite to dissolve organic matter. Int Endod J 2015;48:399–404.

25. Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. J Endod 2004;30:785–7.

26. Estevez R, Conde AJ, Valencia de Pablo O, et al. Effect of passive ultrasonic activation on organic tissue dissolution from simulated grooves in root canals using sodium hypochlorite with or without surfactants and EDTA. J Endod 2017;43:1161–5.

27. De-Deus G, De Berredo Pinho MA, Reis C, et al. Sodium hypochlorite with reduced surface tension does not improve *in situ* pulp tissue dissolution. J Endod 2013;39:1039–43.

28. Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. J Endod 1981;7:376–7.

29. American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 17th ed. Washington D.C.: APHA; 1989.

30. Teruel J, Alcolea A, Hernandez A, Ruiz A. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Arch Oral Biol 2015;60:768–75.

31. Ulusoy ÖI, Savur GI, Alaçam T, Çelik B. The effectiveness of various irrigation protocols on organic tissue removal from simulated internal resorption defects. Int Endod J 2018;51:1030–6.

32. Koskinen KP, Stenvall H, Uitto VJ. Dissolution of bovine pulp tissue by endodontic solutions. Scand J Dent Res 1980;88:406–11.

33. Trepagnier CM, Madden RM, Lazzari EP. Quantitative study of sodium hypochlorite as an *in vitro* endodontic irrigant. J Endod 1977;3:194–6.

34. Boutsioukis C, Lambrianidis T, Kastrinakis E, Bekiaroglou P. Measurement of pressure and flow rates during irrigation of a root canal *ex vivo* with three endodontic needles. Int Endod J 2007;40:504–13.

35. Tejada S, Baca P, Ferrer-Luque CM, et al. Influence of dentine debris and organic tissue on the properties of sodium hypochlorite solutions. Int Endod J 2019;52:114–22.

36. Boutsioukis C, Arias-Moliz MT, Chávez de Paz L. A critical analysis of research methods and experimental models to study irrigants and irrigation systems. Int Endod J 2022;55:295–329.