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ORIGINAL ARTICLE

Effects of continuous chelation on the instrumentation of curved canals with a martensitic NiTi file system: an ex vivo micro-CT evaluation

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Abstract

This study aimed to evaluate the influence of continuous chelation on apical transportation and centrality in shaped curved canals prepared with a martensitic file system and evaluated by micro-CT. Twenty-four lower molar canals with a curvature of 25º–40º were scanned pre-operatively with micro-CT and divided into 2 groups according to two different irrigation regimens (n = 12). Group 1 was irrigated with NaOCl, group 2 with Dual Rinse. All canals were prepared with VDW. ROTATE system. The samples were scanned post-operatively and evaluated at 1, 3 and 5 mm from the apex to determine apical transportation and canal centrality using VG Studio software. Statistical analysis was performed with SPSS software using the non-parametric Mann–Whitney test. No statistically significant differences were found between the two groups for both apical transportation and canal centrality at any of the levels studied (p < 05). The use of continuous chelation during the instrumentation of the canals with martensitic alloy files does not produce greater transportation and does not gener- ate changes in centralization compared to NaOCl-only irrigation. Combined chelation and disinfection while shaping with martensitic NiTi can simplify the irrigation regimen without inducing iatrogenic aberrations.

Keywords Continuous chelation · Curved root canal · Etidronic acid · Micro-CT · Rotate system

Introduction

The aim of endodontic treatment is to shape, disinfect and three-dimensionally obturate the root canal system to prevent bacterial recolonization that could lead to the recurrence of pathology [1].

Maintaining an adequate balance between removal of infected dentine and preservation of the root canal anatomy remains a challenge, particularly in curved canals [2]. Root canal transportation can increase the risk of blockages, strippings, perforations, ledges and ultimately weakening of the tooth structure [3]. Both the transportation and the loss of working length have been directly associated with the alloy stiffness of the mechanical NiTi files utilized [4].

VDW.ROTATE system (VDW, GmbH, Munich, Germany), a second-generation NiTi alloy file system has been recently introduced to the market. It consists of a reduced file sequence compared to its predecessor (MTwo, VDW). According to the manufacturer, VDW.ROTATE has improved flexibility and resistance to cyclic fatigue, due to the inclusion of a heat-treated alloy and minor modifications of the cross-section compared to the MTwo files [5, 6]. The heat treatment induces a predominantly martensitic NiTi alloy within the file characterized by a controlled memory effect, which means that under stress the file can be pre-bent without spring-back effect as for the predominantly austenitic files.

The working blades of the file have a blue tint similarly to the Reciproc Blue, as a result of the oxide layer created after the heat treatment and the grinding of the blank. The shaft of the file has no blue tint being made of a regular non-treated NiTi alloy. Furthermore, it features an off-center design,

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constant taper and a S-shaped cross-section, similarly to the MTwo, that increases its cutting efficiency and decreases the threading-in effect [5].

Endodontic chemo-debridement is achieved associating shaping with irrigating solutions. Sodium hypochlorite (NaOCl) is the most commonly used due to its organic tissue dissolution property and its concomitant antimicrobial action [7]. However, it is not capable of removing inorganic tissue and requires the additional use of chelating solutions [8]. Traditionally, the use of a strong chelator after instrumentation, as penultimate rinse to remove the smear layer, such as 17% ethylenediamine tetra acetic acid (EDTA) solution, has been recommended [9]. Some concerns have been reported in the literature regarding the potential changes in the chemical structure of dentine [10–13], increasing its permeability and solubility, and reducing its microhardness following EDTA usage [14]. Dentin decalcification generated by strong chelators depends on the application time [15], pH and concentration of the solutions [16], supporting a limited use during shaping to avoid over-chelation [11]. However, the recent introduction in the market of weak chelating agents to be mixed with the NaOCl has allowed a simplified irrigation where no alternating of different solutions is required.

The concept of continuous chelation appeared in 2005, and it involves the concurrent use of a NaOCl-containing solution and a chelating agent during the preparation of the root canal system [17]. This has been achieved bind- ing two alkaline chelators (pH range 10.8–12.2) tetraso- dium etidronate (Na4 etidronate) and tetrasodium EDTA (Na4 EDTA) to NaOCl [18, 19]. Maintaining alkalinity maximizes the hypochlorite anion concentration hence the organic tissue dissolution capacity [20].

The continuous chelation presents some advantages such as simplifying and standardizing the irrigation technique and improving the removal of debris from the canal walls [21], enhancing the antimicrobial capacity of NaOCl by dissolving the smear layer [22], promoting the detachment of biofilms from the dentine walls [9] by causing their disruption [23].

Dual Rinse HEDP (Medcem, GMbH, Weinfelden, Switzerland) is the first product to be approved for clinical use in Endodontics. Its presentation consists of a capsule containing 0.9 g of etidronate powder (HEDP), which must be mixed with 10 mL of NaOCl solution immediately before treatment, resulting in a combined solution of active chlorine and 9% HEDP [24].

The influence of continuous chelation used together with heat-treated NiTi alloy instrumentation systems on apical transportation and centralization of the canal has not been studied to date. Therefore, the present study aimed to compare apical transportation and centralization of curved mandibular mesial root canals through instrumentation with VDW.ROTATE system and irrigation with Dual Rinse. The null hypothesis was that weak chelation concurrent to NaOCl irrigation does not yield any differences in apical transportation and canal centrality compared with irrigation carried out with NaOCl irrigation alone.

Materials and methods

This ex vivo randomized trial has been written according to Preferred Reporting Items for Randomized Trials in Endodontics (PRIRATE) 2020 guidelines [25].

Ethical approval was granted by the Ethics and Research Committee of the institution (Universidad Europea de Madrid) where the present ex vivo trial was conducted (CIPI/20/154), subsequently sample calculation was performed to compare two irrigant solutions (NaOCl vs HEDP), using apical transportation and centering ability as three-dimensional parameters.

The total sample size for this study was calculated based on previous research that also used micro-computed tomography (micro-CT) scans to evaluate apical transportation. The Student *t* test for independent samples (Minitab Statistical Software 16.1; Minitab Inc, State College, PA; www.minitab.com) with a D of 7.6, ratio of 1.00, a of 5%, and a power of 80% indicated that the minimum sample size required was 12 samples per group.

Twelve human mandibular first molars that were recently extracted, after gaining verbal informed consent, were selected. The samples were assessed radiographically and by optical microscopy (OPMI pico Dental Microscope, Carl Zeiss, Oberkochen, Germany) to exclude those with root resorption, pre-existing cracks or fractures, calcified or double-curved canals, and open apices. The mesial root length ranged between 20 and 22 mm. Vertucci type IV configuration [26] in the mesial root was mandatory to investigate 2 independent canals individually for all teeth ($n = 24$).

The specimens were cleaned and preserved in a 2% thymol solution prior to the study. The access cavity was done with a diamond bur (number 856, Komet Dental GmBH, Lemgo, Germany). A size 10 k-file (Dentsply Sirona Endodontics) was used to confirm apical patency and establish the working length. The size 10 k-file was passively advanced into the canals until the tip of the instrument had reached the apical foramen. The actual canal length was recorded, and the working length calculated by subtracting 1 mm from this value.

All roots were radiographed, with a 15 k-file in the canal, to establish their angle of maximum curvature as described in previous studies [27]. Their angle of curvature was determined according to Pruett et al. [28] using AutoCad 2014 (Autodesk Inc, San Rafael, CA). This procedure was repeated for both independent mesial canals of each molar.

Samples with angles of curvature between 25º and 40º were included in the study.

A micro-CT scan was performed on all 12 specimens prior to the initiation of the chemomechanical preparation as baseline. For this, an XT H 160 micro-CT device (X-TEC) was used at 20 µ resolution with the XT software package, based on Nikon Metrology's track record of sample flow enhancement. A graphic summary of the methodology is presented in Fig. 1.

Study groups

Stratified sampling was carried out for the division into two study groups ($n = 12$). Apices were sealed with Reus wax to create a closed system [29, 30]. The entire procedure was carried out by immersing the samples in a container with water at 36 ºC.

Group 1: 12 canals (6 mesiolingual and 6 mesiobuccal) were treated. The canals not to be treated were blocked with ISO Tape Teflon (Proclinic). The pulp chambers were filled with 4.25% NaOCl (Dentaflux, Madrid, Spain) and a glide path was performed with size 10 and 15 K-files up to the previously established working length. Afterwards, the basic instrumentation sequence of the VDW.ROTATE system was used (size 15/taper 0.04, size 20/taper 0.05 and size 25/taper 0.06) at 300 rpm and 2.5 N/cm of torque, instrumenting the canal in two steps per file. Each file was used in 2 canals.

Irrigation during instrumentation was performed with a Monoject 27G syringe at 2 mm from the working length with 1.5 mL of 4.25% NaOCl between each instrument. A

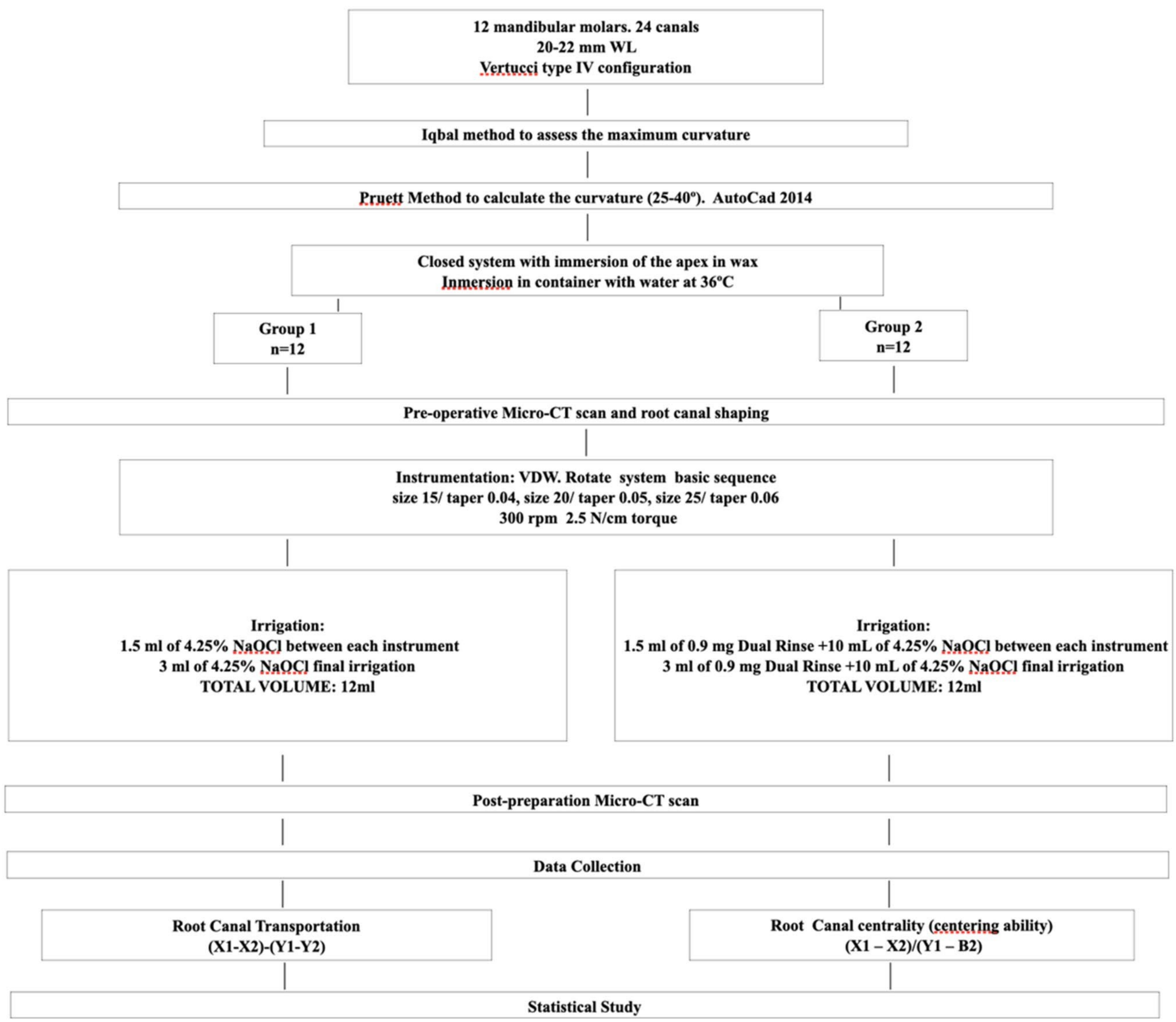

Fig. 1 Flowchart of the methodology

1–2 mm constant apical-coronal movement of the needle was maintained during irrigation. The final irrigation was carried out with 3 mL of the same solution using a total volume of 12 mL per canal.

Group 2: 12 canals (6 mesiolingual and 6 mesiobuccal) were treated. The procedure was identical to the group 1 except that the used irrigant was a solution of 0.9 mg Dual Rinse mixed with 10 mL of 4.25% NaOCl. This solution had to be mixed and replaced within a maximum time of 20 min to avoid losing its properties [31]. The total volume of irrigant used was 12 mL per canal.

The appropriate irrigant for each group was used throughout the shaping phase being flushed and replenished after each file of the sequence to exhaust the full allocated volume by the end of the preparation. Afterwards, the canals were dried with paper points size 25/taper 0.06 (VDW.ROTATE. VDW, GmbH, Munich, Germany) and micro-CT of each tooth was performed with the same parameters as pre-preparation micro-CT.

Micro-CT analysis of apical transportation and centrality

Pre-preparation micro-CT images were analyzed and compared with those generated after the instrumentation and irrigation with the study solution (Fig. 2).

The apical transport analysis was performed at 1, 3 and 5 mm from the working length by measuring canal displacement in millimeters using VG Studio software (Volume Graphics, GmbH, Hexagon), that allows interactive analysis of three-dimensional/volumetric data (voxel data or three-dimensional images). Previous described methodology [32–34] was used to calculate the transportation and the centering ability of the canal. The following formula was used for the calculation of transportation: $(X_1 - X_2) - (Y_1 - Y_2)$, where X_1 corresponds to the shortest distance from the mesial root margin to the canal prior to instrumentation, and X_2 is the shortest distance from the mesial root margin to the prepared canal. The Y_1 and Y_2 measurements are performed in the same way but considering the distal root margin and the canal edge as reference (Fig. 3). Values of 0 indicate no canal transportation whereas any positive or negative value suggests the existence of canal transportation

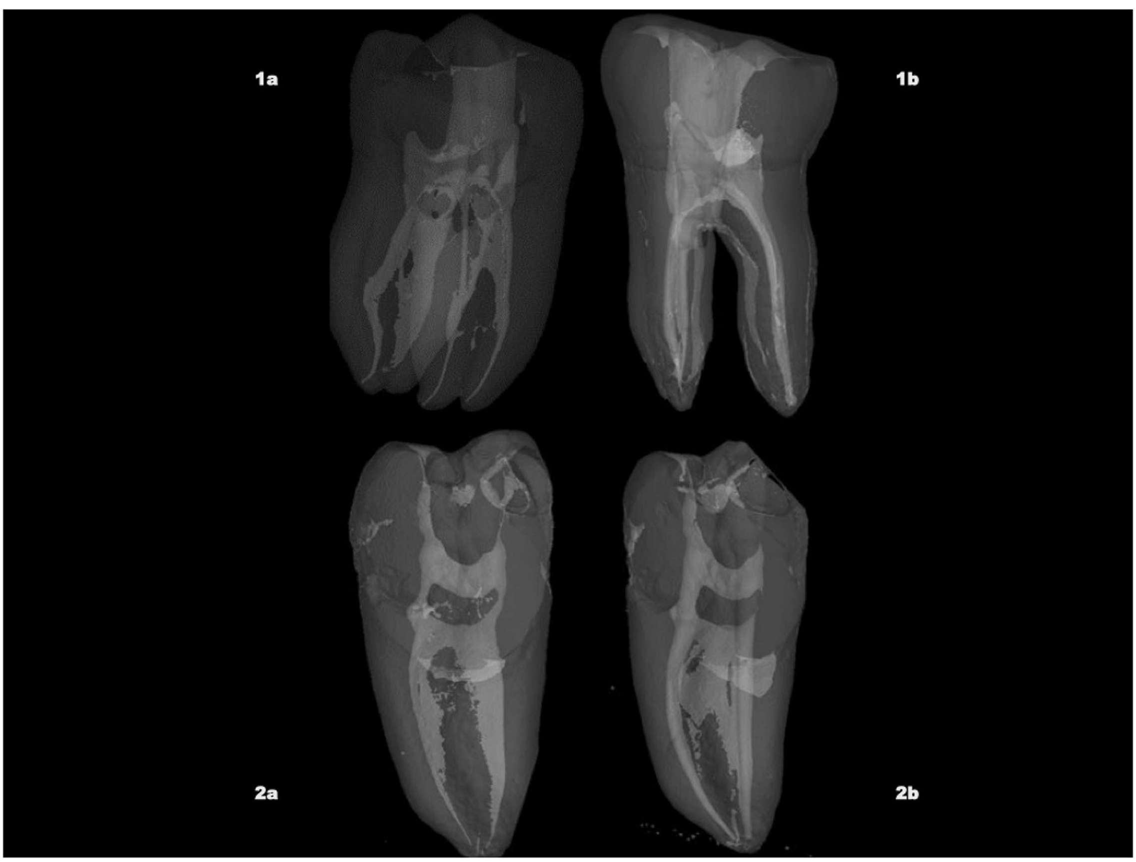

Fig. 2 Representative micro-CT images of teeth before (1a and 2a) and after (1b and 2b) chemomechanical preparation. Group 1 is represented by images 1a and 1b, 2a and 2b represent group 2

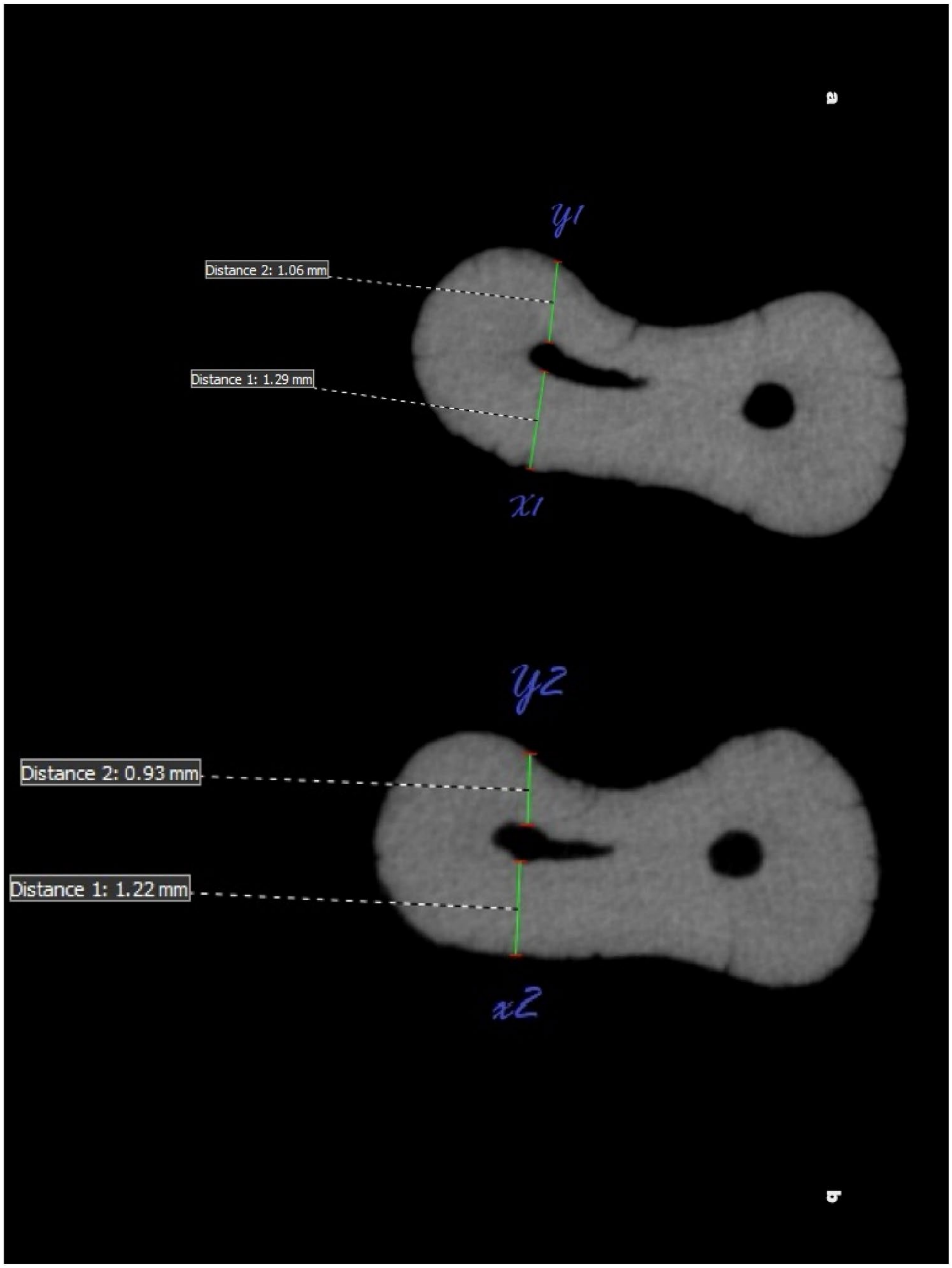

Fig. 3 Micro-CT scan images with markings showing points of measurement before **a** and after **b** preparation

[32–34]. The mean centering ratio indicates the capacity of an instrument to remain centered within the root canal [35]. This was calculated using the following ratio: $(X_1 - X_2)/(Y_1 - Y_2)$ or $(Y_1 - Y_2)/(X_1 - X_2)$. If the obtained results were not equal, the lowest value was considered the numerator of the ratio. The closer the result is to 1, the greater the centering ability of the file within the canal, assuming this result as the optimal centering value.

Statistical analysis

The analysis was made with the statistic software IBM SPSS Statistics 26.0 for Windows. Medians, percentiles 25% and 75, minimum values and maximum values were calculated at 1, 3 and 5 mm. The non-parametric Mann–Whitney *U* test was applied. Differences were set to be statistically significant at $p < 0.05$.

Results

Concerning the apical transportation, no statistically significant difference was found between the study groups at 1, 3 and 5 mm. The data obtained from the median, 25% and 75% percentiles, and the minimum and maximum values are summarized in Table 1.

Table 1 Medians, percentiles, minimum and maximum values for transportation of Group 1 (NaOCl solution) and Group 2 (NaOCl + Dual Rinse) at 1 mm, 3 mm and 5 mm levels

	Group 1				Group 2				*p*
	Median	P25; P75	Min	Max	Median	P25; P75	Min	Max	
1 mm	0.04	−0.01; 0.09	0.01	0.14	0.03	0; 0.05	0.01	0.12	0.04
3 mm	0.01	−0.055	0.02	0.15	0.05	−0.05; 0.06	0.02	0.29	0.01
5 mm	0.035	0.015	0.01	0.16	−0.045	−0.095; 0.01	0.01	0.23	0.035

Table 2 Medians and percentiles values for centrality of Group 1 (NaOCl solution) and Group 2 (NaOCl + dual rinse solution) at 1 mm, 3 mm and 5 mm levels

	Group 1		Group 2		*p*
	Median	P25; P75	Median	P25; P75	
1 mm	2.01	0.70; 3.25	1.42	0.79; 2.83	0.936
3 mm	1.04	0.51; 2.74	1.08	0.51; 3.25	0.857
5 mm	1.28	0.91; 2.18	0.7	0.31; 1.20	0.453

Regarding the centering ability, no statistically significant differences were observed at any level ($p > 0.05$). The results are represented in Table 2.

Discussion

The objective of this study was to analyze the influence of continuous weak chelation in association with martensi- tic instrumentation systems. Although benefits related to reduced debris accumulation during instrumentation with these solutions have been described [17, 36], there is a lack of information regarding their impact on the anatomy of the canal system [37]. The canal transportation may lead to iatrogenic damage or inadequate cleaning of the root canal system resulting in persistence of periradicular lesions [38]. Therefore, assessing the effect of novel irrigation regimens associated with novel instrumentation systems is of paramount importance. For scientific literature, acceptable canal transportation has been defined as between 0.10 mm and 0.30 mm [39, 40]. More extensive transportation could lead to a lack of seal of the root canal filling [39]. In the pre- sent study, data within this range have been obtained for both groups, even though the Dual Rinse group showed a 0.29 mm maximum deviation value, and, therefore, close to the threshold that can be considered as critical.

It should be noted that some studies evaluate transportation using assessment methods that have been described as less accurate than micro-CT analysis [41], which has been considered as highly accurate without the need for sample destruction leading to potential artifacts [42]. Therefore, data obtained through this methodology may be more accurate than those reported by radiography, lacking 3D perspective, or CBCT, due to its lower resolution [32, 33].

The apical transportation and deviation from canal centrality are directly related with instrument stiffness [4]. When these parameters have been evaluated on the predecessor files, MTwo system (similar to VDW.ROTATE system), better results have been obtained when compared to other systems [43], showing to be adequate in maintaining the original canal anatomy. According to the manufacturer, the VDW.ROTATE system has been created to improve the properties of flexibility and resistance to clinical fatigue, by including a heat-treated alloy and small changes in the cross-section with respect to MTwo files. These features should be beneficial to use this file system in curved root canals [5]. In the present investigation, the use of these files has been opted for this reason, as the main objective of the study was to observe the differences related to the irrigation solutions evaluated.

Dual Rinse was selected as continuous weak chelation irrigation regimen, being the only one available on the market to date. Stability of this combined solution is deter- mined by time, NaOCl concentration, and temperature [31, 44], leading to a decrease in free chlorine. Although 60 min of clinical use time at room temperature was ini- tially proposed [44], it has recently been observed that the presence of free chlorine at body temperature decreases substantially after 20 min [31]. Considering that, the aim was to reproduce as much as possible a real clinical situ- ation simulating body temperature at 36 °C, the renewal of the solution has been adjusted to the times described (20 min) in the present study.

To prevent the irrigant solution from entering the untreated canal, the access was blocked with Teflon, and prepared individually. This avoids a laboratory carry-over effect with longer contact time of a chelating agent on the dentine structure. At the same time, the volume of irrigant used has been standardized to 12 mL. Some investigations have advised caution on the application of irrigants to remove the smear layer regarding their potential to produce changes in the chemical structure of dentine [10–13]. The volume used for irrigation is intimately related to the exposure time of the dentine to the irrigants, being a fact to consider. In a previous study [37], the authors used 15 mL of the irrigant solutions, presumably because of the increased number of instruments used for the canals preparation com- pared to the present investigation (ProTaper Universal vs VDW.ROTATE).

To the authors' knowledge, only one investigation evaluating the influence of chelating solutions on canal transportation has been published [37]. Conversely to this study, the authors observed greater canal transportation when chelating substances such as etidronate were used. It is possible that the disparity in the results is based on methodological differences. In the study by Silva e Souza et al., the differences between using continuous chelation with etidronic, and using EDTA and NaOCl alternately, compared to a control group with saline, were evaluated [37]. The etidronic group obtained the worst results, and the authors speculated that this may have been due to the longer irrigation time of this solution, without specifying the exposure time. In the present work, the shaping and irrigation time interval was standardized to 3 min per canal. In a clinical setting, when root canal treatment is carried out on a multi-rooted tooth, the irrigant may overflow and be in contact with the dentine walls of the canals not being instrumented prolonging the overall exposure to the chelating agent. This chelation effect with weak chelating agents is negligible in the absence of instrumentation as the irrigant penetration per se is self-limiting as previously shown [13].

In Silva e Souza et al. study, the control group was irrigated with saline, whereas in the present investigation, NaOCl was used [37]. Furthermore, these authors did not describe the angles of curvature of the samples and their distribution, potentially leading to sample selection bias. On the other hand, Silva and Souza used an austenitic file system (ProTaper Universal), which may have caused a greater transportation due to the higher spring-back effect and, therefore, accounting for the differences with our findings. Finally, the method of evaluation may also have had an influence, as the samples were analyzed with two-dimensional radiography as opposed to micro-CT.

Future lines of research should evaluate the influence of other types of martensitic alloys (e.g., Gold or CM-wire) for curvatures above 40º in combination with continuous chelation during controlled times of exposition, using a standardized methodology, to add more information and overcome the limitations of this in vitro study.

Conclusions

Under the limitations of this study, NaOCl irrigation in combination with etidronic acid during instrumentation does not increase canal transportation at 1, 3 and 5 mm from the apex, nor deviation from canal preparation centrality compared with a conventional NaOCl irrigation regimen. Further studies are needed to determine the effect of etidronic acid irrigation in combination with other different instrumentation systems as based on the present study, the results have been proven only for the instruments investigated and thus may not simply be generalized as fundamental.

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Declarations

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval Ethical approval was granted by the Ethics and Research Committee of the institution (Universidad Europea de Madrid) where the present ex vivo trial was conducted (CIPI/20/154).

Informed consent Verbal informed consent was obtained from the participants recruited in the study.

References

1. Stewart GG. The importance of chemomechanical preparation of the root canal. Oral Surg Oral Med Oral Pathol. 1955;8:993–7. https://doi.org/10.1016/0030-4220(55)90303-0.
2. Kunert GG, Fontanella VRC, de Moura AAM, Barletta FB. Analysis of apical root transportation associated with protaper universal F3 and F4 instruments by using digital subtraction radiography. J Endod. 2010;36:1052–5. https://doi.org/10.1016/j.joen.2010.02.004.
3. Franco V, Fabiani C, Taschieri S, Malentacca A, Bortolin M, Del Fabbro M. Investigation on the shaping ability of nickel-titanium files when used with a reciprocating motion. J Endod. 2011;37:1398–401. https://doi.org/10.1016/j.joen.2011.06.030.
4. Javaheri H, Javaheri G. A comparison of three Ni-Ti rotary instruments in apical transportation. J Endod. 2007;33:284–6. https://doi.org/10.1016/j.joen.2006.05.004.
5. Ertuğrul İF, Orhan EO. Cyclic fatigue and energy-dispersive X-ray spectroscopy examination of the novel ROTATE instrument. Microsc Res Tech. 2019;82:2042–8. https://doi.org/10.1002/jemt.23374.
6. Uslu G, Gundogar M, Özyurek T, Plotino G. Cyclic fatigue resistance of reduced-taper nickel-titanium (NiTi) instruments in doubled-curved (S-shaped) canals at body temperature. J Dent Res Dent Clin Dent Prospects. 2020;14:111–5. https://doi.org/10.34172/joddd.2020.024.
7. BystrÖM A, Sunvqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J. 1985;18:35–40. https://doi.org/10.1111/j.1365-2591.1985.tb00416.x.
8. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216:299–303. https://doi.org/10.1038/sj.bdj.2014.204.
9. Zehnder M. Root canal irrigants. J Endod. 2006;32:389–98. https://doi.org/10.1016/j.joen.2005.09.014.
10. Dogan H, Calt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. J Endod. 2001;27:578–80. https://doi.org/10.1097/00004770-200109000-00006.
11. Rajasingham R, Ng YL, Knowles JC, Gulabivala K. The effect of sodium hypochlorite and ethylenediaminetetraacetic acid irrigation, individually and in alternation, on tooth surface strain. Int Endod J. 2010;43:31–40. https://doi.org/10.1111/j.1365-2591.

2009.01625.x.

12. Ng YL, Reddington LP, Berman A, Knowles JC, Nazhat SN, Gulabivala K. Viscoelastic and chemical properties of dentine after different exposure times to sodium hypochlorite, ethylenediaminetetraacetic acid and calcium hydroxide. Aust Endod J. 2020;46:234–43. https://doi.org/10.1111/aej.12397.
13. Ramírez-Bommer C, Gulabivala K, Ng YL, Young A. Estimated depth of apatite and collagen degradation in human dentine by sequential exposure to sodium hypochlorite and EDTA: a quantitative FTIR study. Int Endod J. 2017;51:469–78. https://doi.org/10.1111/iej.12864.
14. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. J Endod. 2004;30:792–5. https://doi.org/10.1097/01.don.0000128747.89857.59.
15. Sobhani OE, Gulabivala K, Knowles JC, Ng YL. The effect of irrigation time, root morphology and dentine thickness on tooth surface strain when using 5% sodium hypochlorite and 17% EDTA. Int Endod J. 2010;43:190–9. https://doi.org/10.1111/j.1365-2591.2009.01655.x.
16. Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP, Martín-Peinado FJ, González-López S. Decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine. Int Endod J. 2008;41:418–23. https://doi.org/10.1111/j.1365-2591.2007.01371.x.
17. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod. 2005;31:817–20. https://doi.org/10.1097/01.don.0000158233.59316.fe.
18. Tartari T, Oda DF, Zancan RF, da Silva TL, de Moraes IG, Duarte MAH, Bramante CM. Mixture of alkaline tetrasodium EDTA with sodium hypochlorite promotes in vitro smear layer removal and organic matter dissolution during biomechanical preparation. Int Endod J. 2016;50:106–14. https://doi.org/10.1111/iej.12595.
19. Tartari T, Guimarães BM, Amoras LS, Duarte MAH, SilvaeSouza PAR, Bramante CM. Etidronate causes minimal changes in the ability of sodium hypochlorite to dissolve organic matter. Int Endod J. 2014;48:399–404. https://doi.org/10.1111/iej.12329.
20. Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci. 2006;11:147–57. https://doi.org/10.4265/bio.11.147.
21. Paqué F, Rechenberg D-K, 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. https://doi.org/10.1016/j.joen.2011.12.019.
22. Morago A, Ruiz-Linares M, Ferrer-Luque CM, Baca P, Rodríguez Archilla A, Arias-Moliz MT. Dentine tubule disinfection by different irrigation protocols. Microsc Res Tech. 2019;82:558–63. https://doi.org/10.1002/jemt.23200.
23. Arias-Moliz MT, Morago A, Ordinola-Zapata R, Ferrer-Luque CM, Ruiz-Linares M, Baca P. Effects of dentin debris on the antimicrobial properties of sodium hypochlorite and etidronic acid. J Endod. 2016;42:771–5. https://doi.org/10.1016/j.joen.2016.01.021.
24. Zollinger A, Mohn D, Zeltner M, Zehnder M. Short-term storage stability of NaOCl solutions when combined with dual rinse HEDP. Int Endod J. 2017;51:691–6. https://doi.org/10.1111/iej.12875.
25. Nagendrababu V, Murray PE, Ordinola-Zapata R, Peters OA, Rôças IN, Siqueira JF, Priya E, Jayaraman J, Pulikkotil S, Camilleri J, et al. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: a consensus-based development. Int Endod J. 2021;54:1482–90. https://doi.org/10.1111/iej.13542.
26. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol. 1984;58:589–99. https://doi.org/10.1016/0030-4220(84)90085-9.
27. Iqbal MK, Maggiore F, Suh B, Edwards KR, Kang J, Kim S. Comparison of apical transportation in four Ni-Ti rotary

instrumentation techniques. J Endod. 2003;29:587–91. https://doi.org/10.1097/00004770-200309000-00011.

28. Pruett JP, Clement DJ, Carnes DL Jr. Cyclic fatigue testing of nickel-titanium endodontic instruments. J Endod. 1997;23:77–85. https://doi.org/10.1016/S0099-2399(97)80250-6.
29. Parente JM, Loushine RJ, Susin L, Gu L, Looney SW, Weller RN, Pashley DH, Tay FR. Root canal debridement using manual dynamic agitation or the EndoVac for final irrigation in a closed system and an open system. Int Endod J. 2010;43:1001–12. https://doi.org/10.1111/j.1365-2591.2010.01755.x.
30. de Gregorio C, Arias A, Navarrete N, del Rio V, Oltra E, Cohenca N. Effect of apical size and taper on volume of irrigant delivered at working length with apical negative pressure at different root curvatures. J Endod. 2013;39:119–24. https://doi.org/10.1016/j.joen.2012.10.008.
31. Wright PP, Kahler B, Walsh LJ. The effect of heating to intracanal temperature on the stability of sodium hypochlorite admixed with etidronate or EDTA for continuous chelation. J Endod. 2019;45:57–61. https://doi.org/10.1016/j.joen.2018.09.014.
32. Hasheminia SM, Farhad A, Sheikhi M, Soltani P, Hendi SS, Ahmadi M. Cone-beam computed tomographic analysis of canal transportation and centering ability of single-file systems. J Endod. 2018;44:1788–91. https://doi.org/10.1016/j.joen.2018.09.011.
33. Gergi R, Rjeily JA, Sader J, Naaman A. Comparison of canal transportation and centering ability of twisted files, Pathfile-ProTaper system, and stainless steel hand k-files by using computed tomography. J Endod. 2010;36:904–7. https://doi.org/10.1016/j.joen.2009.12.038.
34. Nevares G, de Albuquerque DS, Freire LG, Romeiro K, Fogel HM, dos Santos M, Cunha RS. Efficacy of ProTaper NEXT compared with reciproc in removing obturation material from severely curved root canals: a micro-computed tomography study. J Endod. 2016;42:803–8. https://doi.org/10.1016/j.joen.2016.02.010.
35. Gambill JM, Alder M, del Rio CE. Comparison of nickel-titanium and stainless steel hand-file instrumentation using computed tomography. J Endod. 1996;22:369–75. https://doi.org/10.1016/ s0099-2399(96)80221-4.
36. De-Deus G, Zehnder M, Reis C, Fidel S, Fidel RAS, Galan J, Paciornik S. 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. https://doi.org/10. 1016/j.joen.2007.09.020.
37. SilvaeSouza PAR, das Dores RSE, Tartari T, Pinheiro TPS, Tuji FM, SilvaeSouza MH. Effects of sodium hypochlorite associated with EDTA and etidronate on apical root transportation. Int Endod J. 2013;47:20–5. https://doi.org/10.1111/iej.12118.
38. Pettiette MT, Delano EO, Trope M. Evaluation of success rate of endodontic treatment performed by students with stainless-steel K-files and nickel-titanium hand files. J Endod. 2001;27:124–7. https://doi.org/10.1097/00004770-200102000-00017.
39. Wu M, Fan B, Wesselink P. Leakage along apical root fillings in curved root canals. Part I: effects of apical transportation on seal of root fillings. J Endod. 2000;26:210–6. https://doi.org/10.1097/ 00004770-200004000-00003.
40. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004;30:559–67. https:// doi.org/10.1097/01.don.0000129039.59003.9d.
41. de Sousa-Neto MD, Silva-Sousa YC, Mazzi-Chaves JF, Carvalho KKT, Barbosa AFS, Versiani MA, Jacobs R, Leoni GB. Root canal preparation using micro-computed tomography analysis: a literature review. Braz Oral Res. 2018. https://doi.org/10.1590/ 1807-3107bor-2018.vol32.0066.
42. Paqué F, Ganahl D, Peters OA. Effects of root canal preparation on apical geometry assessed by micro-computed tomography. J

Endod. 2009;35:1056–9. https://doi.org/10.1016/j.joen.2009.04.020.
43. Shivashankar MB, Niranjan NT, Jayasheel A, Kenchanagoudra MG. computed tomography evaluation of canal transportation and volumetric changes in root canal dentin of curved canals using Mtwo, ProTaper and ProTaper next rotary system—an in-vitro study. J Clin Diagn Res. 2016;10:ZC10–4. https://doi.org/10.7860/JCDR/2016/20373.8788.
44. 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. https://doi.org/10.1016/j.joen.2016.12.006.