# Efficacy on cleansing post space root canal walls after Hydraulic Endodontic Sealers were used



#### Abstract

#### Aim of the study

The aim of this study was to evaluate the efficacy on the cleansing of root walls from several hydraulic endodontic sealers, of smear layer, of the debris, and gutta-percha after the preparation of the restorative space.

#### Methods

Sixty single rooted have been collected. All the samples were prepared by the same operator (DIKP), using Nickeltitanium rotating instruments (MTwo) through the Simultaneous Shaping Technique. The Continuous-wave of condensation technique of obturation was used in combination with the tested hydraulic endodontic sealers. To all specimens, the restorative space has been made, leaving 5 mm of apical guttapercha and performed postoperative periapical X-Rays. The samples were randomly divided into 4 groups: Group A: traditional endodontic sealer; Group B): Experimental GC hydraulic sealer; Group C: OneFill hydraulic sealer; Group 4: Ceraseal hydraulic sealer. Accordingly with the used cleansing procedure, the 4 Groups were subdivided in 3 SubGroups: A: no cleansing after preparation of root canal space; B: Cleansing with EDTA 17%;

C: Cleansing with ETDA 17% ultrasonic activated. All dental sample groups were cut longitudinally with a low-speed saw (Isomet); the samples were observed using a scanning electron microscope (Jeol, Jsm-6060LV) to evaluate: 1. Amount of debris/smear layer; 2. Amount of obstruction of dentinal tubules found in the groups; 3. Evaluation of the presence of hydraulic endodontic sealer. Statistical analysis was performed by Mann-Whitney non-parametric comparison test.

#### Results

The SEM observations showed that to clean the root canal wall from hydraulic sealer EDTA 17% activated with ultrasonic device are needed. The traditional sealer was statistically significant better cleaned from root canal walls than hydraulic sealers.

#### Conclusions

The hydraulic endodontic sealers are cleaned with more difficulty than traditional endodontic sealer.

#### **Clinical significance**

The hydraulic endodontic sealers can be an obstacle to adhesive reconstruction of endodontically treated teeth.

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Post space, Cleaning root canal, Ultrasonic activation.

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## **INTRODUCTION**

Recently hydraulic endodontic sealers were proposed into the market and become more and more popular among both general practitioners and endodontists. This new family of endodontic sealers has osteo-inductive capacity, high flowability into lateral canals and the dentinal tubules as well and can be used in combination of single cone and/or warm gutta-percha obturation technique (1-10). When used in combination with the single cone technique the thickness of the sealer into the root canal is pretty high while when used with a warm gutta-percha technique it can be much thinner (1,4,8,9). The removal procedure of hydraulic endodontic sealers from the root canal is still under investigation and it is needed when a retreatment can be made and/or when an endodontic space must be prepared to restore the root. Also, the hydraulic endodontic sealer, after being set, become a rigid material and it can be difficult to remove it from the root canal at the time of the root canal space preparation for an adhesive restoration (1,2). An adhesive system needs a clean substrate to adhere and open tubules' orifices to flow and guarantee the material penetration into the tubules and, after being set, resin tags and hybrid layer formation, that are key factors of the micromechanical bonding mechanism to dentin (11). The wide majority of root canal obturation is still made using a zinc phosphate sealer. The preparation of the restorative space into the root canal determines the formation of a smear layer that can also plug the tubules' orefices with smear plugs (11). The smear layer must be completely removed before bonding procedure to optimize it (11). In order to achieve the highest bond strength to dentin and its better seal, a cleaned bonding surface must be available and there was a long discussion about which one is the best irrigation solution and procedure for this purpose (12,13). Pretty often sodium hypochlorite (NaOCl) was utilized as an irrigation solution but could affect the bond strength of the cement with the root canal dentin (14). Although manufacturers recommend NaOCl as a cleaner of the space, the above-mentioned procedure can adversely affect the resin-cement bond strength (14). Other studies, instead, were showing that alternating irrigation with NaOCl and ethylenediaminetetraacetic acid (EDTA) can completely remove the smear layer, increase the adhesive penetration and in the end, increase the bond strength of the post cement to the dentin (15-16).

The use of ultrasonic device to increase the effectiveness of irrigant solutions is well accepted although not all general practitioners use it routinely (17).

The adhesives are currently available on the market to restore an endodontic treated root range from totaletch, self-etch, to self-adhesive systems (12). It is believed that a wide number of practitioners, after preparing the restorative space into the root canal, just applied the etching/bonding system without any other additional cleaning-irrigating steps. For the radicular posts cementation the resin-based types of cement are the material of choice (18-19): in the literature have been reported good clinical performances and high rates of success for teeth restored with fiber posts in conjunction with several resin types of cement and adhesive systems (19-21). Despite that, the evidence gathered from clinical trials shows that post cement removal is one of the most common failure modes (22). Most of the time is the dentin-resin-cement interface the weakest point and consequently more frequently subject to failure (20-22). The above-mentioned interface could be influenced by several factors, like the dentine state, or the dentinal tubules orientation, or the irrigation solution used, depth of the intra-radicular area, the type of adhesive system, and the type of endodontic cement used (23).

The aim of this study was to evaluate the role of EDTA alone and activated by ultrasonic device on the cleansing of root walls, of the smear layer, of the debris, and guttapercha after the preparation of the restorative space. The null hypothesis tested were that:

1. there was no difference on using or not the EDTA and 2. there was no difference on using ultrasonic device or not.

## **MATERIALS AND METHODS**

## **Samples preparation**

One hundred and twenty dental single roots (n=120) have been collected. Extraction was done for periodontal reasons and preoperative periapical X-rays were performed. Teeth preservation was done using physiological saline solution. The access cavity was performed with a cylindrical diamond bur 206, an endodontic probe (DG16, Hu-Friedy, USA) was used to locate the canal openings, lastly a patency file K-file 08/10/15 has been utilized for reaching the working length and maintaining root canals patency. The endodontic treatment was performed through a disinfection protocol chemo-mechanic standard with proper irrigants (16). All the samples were prepared by the same operator, using Nickel-titanium (Ni-Ti) rotating instruments (Mtwo, Sweden & Martina, Le Due Carrare, Italy) through the Simultaneous Shaping Technique where four instruments were used in sequence: 10.04 at WL, 15. 05 at WL, 20.05 at WL, 25.06 at WL.

The use of the mechanical instruments was alternated with irrigation cycles with 2 mL of. NaOCl al 5,25% at room temperature, then apical gauging was performed utilizing a Ni-Ti hand K-file (NiTiflex, Maillefer, Bailague, Switzerland) with the same size of the last apical rotating instrument used, at the end of the procedure 5ml of EDTA 17% (OGNA Pharmaceutical laboratories, Milano, Italy) where used to irrigate the canal and left for two minutes. The final rinse was done with 5 mL di NaOCl at 5, 25% (OGNA Pharmaceutical laboratories, Milano, Italy); afterwards the gutta-percha cone-fit was performed

with dedicated gutta-percha cones (Mtwo, Sweden & Martina, Le Carrare, Italy). The root canals were dried out using paper points and three-dimensionally obturated with guttapercha and a ZOE sealer (Argoseal, Ogna Pharmaceutical laboratories, Milano, Italy) as cement, with the continuous-wave of condensation technique of obturation using a SytemB/Sybron Endo as a device for the down filling, for the backfilling of the middle third and coronal third has been utilized the Obtura Syringe (Metasystems EQ-V, Sweden & Martina, Le Due Carrare, Italy). To all specimens, post-operative periapical X-Rays were performed, sub sequentially the restorative space has been made with cutters Gates Glidden N° 2, N°3, leaving 5 mm of apical gutta-percha and performed once more post-operative periapical X-Rays. Based upon the treatment used the samples were randomly divided in 4 groups and 3 subgroups each group accordingly the hydraulic endodontic sealer used and the cleaning procedure was applied: Group 1: Control (Giovanni Ogna & Figli, Muggio, MB, Italy); Group 2: Experimental GC (Trial Bio-ceramic Sealer NBS-162, GC Co, Tokyo, Japan); Group 3: Onefill (MDClus, Chungbuk, Korea); Group 4: Ceraseal (Meta Biomed, Chunbuk, Korea).

The SubGroups were as follow: SubGroup A: The root canals were prepared using a premade drill and then was washed with an endodontic syringe and distilled water after being etched with phosphoric acid at 37% for 20 seconds. SubGroup B: The root canals after being prepared as in Group 1 were cleansing of the root walls with ultrasonic activation of the irrigant (Newtron P5 XS; Satelec Acteon, Norwich, UK) with distilled water. SubGroup C: The root canals after being prepared as in Group 1 were cleansing of the root walls with ultrasonic activation of the irrigant sing of the root walls with ultrasonic activation of the root walls with ultrasonic activation of the root walls with ultrasonic activation of the irrigants (Newtron P5 XS; Satelec Acteon, Norwich, UK) with EDTA at 17%.

Then, the dental sample groups were cut longitudinally with a low-speed saw (Isomet, Buehler Co., USA). The samples were cut not completely and then fractured with a blad in order to avoid any contamination of the root canal space. Selected sections were mounted on matrices and goldsputtered using a sputtering device (Emitech K550, Quorum, Laughton, UK). After that, the samples were observed using a scanning electron microscope (Jsm-6060LV Scanning Electron Microscope, Jeol, Tokyo, Japan) at different magnifications. Images at different magnifications (100x, 1000x, 2500x, and 10000x) were made on coronal third, medium third and apical third of root canal preparation, and observed separately according to the following criteria (Figs 1A-1D):

## Amount of debris/ smear layer

The amount of smear layer or plugs on the tooth surface has been evaluated on the basis of observation of pictures taken using a microscope at different degrees of magnifications (10x), where the rating scale is between 1 and 5.

- 1: more or equal to 50%;
- 2: less or equal to 40%;
- 3: less or equal to 30%;
- 4: less or equal to 20%;
- 5: less or equal to 10%.

## Amount of obstruction of dentinal tubules found in the groups

The researcher through observational studies has evaluated the amount of obstructed tubules with a rating scale from 1 to 5, the scale is the same as the one utilized previously for the debris evaluation (Figs 2a-2b).

The "amount of obstructed dentin tubules" were divided from 1 to 5:

- » 1: more or equal to 90%;
- » 2: less or equal to 70%;
- » 3: less or equal to 50%;
- » 4: less or equal to 20%;
- » 5: less or equal to 10%.

## Presence of piece sealer/gutta-percha

The presence or not of visible piece of endodontic sealer and/or gutta-percha was after cutting the



Fig. 1A Root canal preparation, Group 1 (Control Group) (x10); 1B: Root canal preparation: coronal third (x33); 1C: Root canal preparation: medium third (x33); 1D: Root canal preparation: apical third (x33).





**Fig. 2A** Example of score 5. Group 1 (Control Group): magnification of dentinal wall (x500) after being well cleaned from endodontic sealer, smear layer and smear plugs. The dentinal wall looks cleaned and tubules are detectable.



**Fig. 2B** Example of score 1. Root canal preparation. Group 1 (Control Group): magnification of dentinal wall (x1000) after being not cleaned from endodontic sealer, smear layer and smear plugs. A layer remains on top of dentinal wall covering it.



Fig. 3A Sub Group A Control. Traditional sealer. SubGroup A: Magnification of dentinal wall ( $\times$ 1000). It is not possible to note any open tubule, in all 4 Groups.



Fig. 3C Sub Group A Control. One fill. SubGroup A: Magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



 $Fig.\ 3B$  Sub Group A Control. Exp GC. SubGroup A: Magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



Fig. 3D Sub Group A Control. Ceraseal. SubGroup A: Magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



sample roots. The presence or not of pieces of material into the root canal was simply recorded.

The microscopic observations were made by two calibrated expert operators: in case of different evaluations, the two operators re-evaluated together the microscopic picture and found an agreement. The amount of samples with different ratings for the presence of debris as well as the amount of open dentinal tubules was assessed. A non-parametric test for multiple-group comparison (Kruskal-Wallis) was used to test two null hypotheses:

- The scores for the percentage of debris on the 1. tooth surface and for the percentage of open dentinal tubules were not different within samples of the same endodontic cement cleansed with the three different strategies (i.e., normal irrigation, EDTA, EDTA + ultrasounds).
- 2. The scores for the percentage of debris on the tooth

Fig. 4A Sub Group B EDTA. Traditional sealer. SubGroup B: Magnification of dentinal wall (x1000). It is possible to note any tubules but not really opened, in all 4 Groups.

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opened, in all 4 Groups.

Fig. 4C Sub Group B EDTA.. One fill. SubGroup B: Magnification of

dentinal wall (x1000). It is possible to note any tubules but not really

surface and for the percentage of open dentinal tubules were not different between samples of different endodontic cements cleansed with the same strategy.

Statistical significance was set at p<0.01.

## RESULTS

A view at different magnifications of all four Groups and each three subgroups is shown.

The results of SEM observation of the samples treated with or without ultrasounds and or EDTA are reported below. All the 4 tested Groups showed that only the preparation of the root canal with a premade drill followed by orthophosphoric application can't effectively remove the traditional and hydraulic endodontic sealers (SubGroups A). A layer of hydraulic sealer remained to cover the dentinal walls and

Fig. 4B Sub Group B EDTA. Exp GC. SubGroup B: Magnification of dentinal wall (x1000). It is possible to note any tubules but not really opened, in all 4 Groups

Fig. 4D Sub Group B EDTA. Ceraseal. SubGroup B: Magnification of dentinal wall (x1000). It is possible to note any tubules but not really opened, in all 4 Groups.









consequently tubules were not exposed at all (Figs. 3A-3D ).

Ultrasound treatment with distilled water showed a minimum capacity to remove the sealer from the root canal walls but exposures of dentinal tubules was still limited (SubGroups B)(Figs. 4A-4D).

When the ultrasound treatment was combined with a 17% EDTA solution for one minute all root samples showed a better removal of endodontic sealers with tubules exposure (SubGroups C)(Figs. 5A-5D).

However in Group 2-4 samples, at higher magnification, the tubules did not appear to be cleaned and opened and some debris remained covering intertubular dentin (Figs. 6A-6C). The presence of visible piece of endodontic sealer/gutta-percha was found in all groups. Inside each Group of hydraulic endodontic sealers, it was evident that from SubGroups A to C the cleansing procedures were more effective (Figs 7A-7C



Fig. 5A Sub Group C EDTA and ULTRASOUND. Traditional sealer. SubGroup C: Magnification of dentinal wall (x1000). It is possible to note open tubules, in all 4 Groups.



Fig. 5C Sub Group C EDTA and ULTRASOUND. One fill. SubGroup C: Magnification of dentinal wall (x1000). It is possible to note open tubules, in all 4 Groups.

and 8A-8C).

The amount of debris is not satisfactory in SubGroup A, while SubGroups B and C which has been treated with ultrasounds showed better result in most of the samples. The importance and value on removing the hydraulic endodontic sealers by the ultrasound treatment in combination with 17% EDTA solution was evident in all Groups. However, the complete cleaning of sealer/debris and opening of tubules observed in Group 1, was never reached when hydraulic endodontic sealers were used.

The Kruskal-Wallis test allowed rejecting all null hypotheses (p<.001) (Tables 1,2):

- 1. The percentage of debris covering the tooth surface was different within samples of the same cement cleansed with different strategies, with EDTA + Ultrasound being the most effective strategy.
- 2. The percentage of open dentinal tubules was



Fig. 5B Sub Group C EDTA and ULTRASOUND. Exp GC. SubGroup C: Magnification of dentinal wall (x1000). It is possible to note open tubules, in all 4 Groups.



Fig. 5D Sub Group C EDTA and ULTRASOUND. Ceraseal. SubGroup C: Magnification of dentinal wall (x1000). It is possible to note open tubules, in all 4 Groups.



Fig. 6A Sub Group A Prep. Exp GC. SubGroup A: High magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



Fig. 7A Sub Group A Prep. Exp GC. SubGroup A (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable.



 $Fig.\ 6B$  Sub Group A Prep. One fill. SubGroup A: High magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



Fig. 7B Sub Group B EDTA. Exp GC. SubGroup B. (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable.



**Fig. 6C** Sub Group A Prep. Ceraseal. SubGroup A: High magnification of dentinal wall (x1000). It is not possible to note any open tubule, in all 4 Groups.



Fig. 7C Sub Group C EDTA and ULTRASOUND. Exp GC. SubGroup C. (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable. In this picture the tubule is opened, the intertubular dentin cleaned and small debris of bioceramic sealer still present.





Fig. 8A Sub Group A Prep. One fill. SubGroup A (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable.



Fig. 8B Sub Group B EDTA. One fill. SubGroup B. (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable. However, also after EDTA and ULTRASOUND, the dentin is not completely cleaned.



**Fig. 8C** Sub Group C EDTA and ULTRASOUND. One fill. SubGroup C. (x1000). It is possible to see how, changing the clinic procedure, the tubule become more detectable. However, also after EDTA and ULTRASOUND, the dentin is not completely cleaned.

different within samples of the same cement cleansed with different strategies, with EDTA + Ultrasound being the most effective strategy.

- 3. The percentage of debris covering the tooth surface was different between samples of different cements cleansed with the same strategy, with cement #1 being the easiest to clean.
- 4. The percentage of open tubules was different between samples of different cements cleansed with the same strategy, with cement #1 being the easiest to clean.

## DISCUSSION

In this study three hydraulic endodontic sealers were used in combination with the warm gutta-percha obturation technique. Recently several authors showed good clinical outcomes and very positive in vitro results when these new sealers are used with warm gutta-percha obturation technique (4,9,24). However, similar results of in vivo and in vitro tests are available when hydraulic endodontic sealers are used with single cone obturation technique (25-27). In this study the warm gutta-percha obturation technique was used to have a thinner thickness of sealer to be removed. Similar studies using hydraulic endodontic sealers in combination with the single cone obturation technique are on the road. The restoration of a non-vital tooth with or without the insertion of a fiber post and resin materials is a common clinical procedure in dentistry. The adhesion to the dentinal tissue is due to micromechanical retentions made on the demineralized dentin surface and through the formation of a hybrid layer and resin tags (11-13), therefore the post-space cleansing is really mandatory and can influence the bond strength of the post to the dentin (13). There are many adhesive systems available in the market. Some authors argue that using self-etch has advantages over the total-etch ones, because they do not require a moist dentin substrate that is difficult to control within the canal (27), furthermore the selfetch adhesive strength is not affected by the post-space depth (28-30). However, after the preparation of the restorative-space, the canal walls are characterized by the presence of the thick and sticky smear layer, and putting in place the self-etch system will not be straight forward (31), and that is why total-etch systems are considered better in the adhesion process due to the etching effect of orthophosphoric acid while removing the smear-laver from the canal walls (31). In the control group (Group 1) of this study orthophosphoric acid was applied in order to reproduce the procedure of bonding widely performed by general practitioner. From the observed results of this in vitro study, it can be considered that the simple application of orthophosphoric acid at 37% for 20 seconds was not sufficient to remove the remained hydraulic endodontic sealer from the root canals.



	Cement 1 (GC Experimental)	Cement 2 (One Fill)	Cement 3 (Ceraseal)	Cement 4 (Traditional)	Between- cement diff.
Control	Score 1: N=10	Score 1: N=10	Score 1: N=10	Score 1: N=10	H=0, P<.001
EDTA	Score 4: N=1	Score 2: N=10	Score 2: N=3	Score 2: N=2	H=28.2, P<.001
	Score 5: N=9		Score 3: N=7	Score 3. N=5	
				Score 4: N=3	
EDTA + US	Score 5: N=10	Score 3: N=4	Score 3: N=4	Score 4: N=8	H=20.8, P<.001
		Score 4: N=5	Score 4: N=6	Score 5: N=2	
		Score 5: N=1			
Within-cement diff.	H=19.4, P<.001	H=25.8, P<.001	H=22.6, P.001	H=23.8, P<.001	

Tab. 1 Evaluation of debris presence after cleansing. Comparison of different cleansing strategies and sealers. Differences: Mann-Whitney scores

	Cement 1 (GC Experimental)	Cement 2 (One Fill)	Cement 3 (Ceraseal)	Cement 4 (Traditional)	Between- cement diff.
Control	Score 1: N=10	Score 1: N=10	Score 1: N=10	Score 1: N=10	H=0, P<.001
EDTA	Score 4: N=9	Score 3: N=1	Score 1: N=3	Score 2: N=2	H=27.1, P<.001
	Score 5: N=1	Score 4: N=7	Score 2: N=4	Score 3. N=7	
		Score 5: N=2	Score 3: N=3	Score 4: N=1	
EDTA + US	Score 5: N=10	Score 4: N=2	Score 3: N=2	Score 4: N=7	H=16.4, P<.001
		Score 5: N=8	Score 4: N=7	Score 5: N=3	
			Score 5: N=1		
Within-cement diff.	H=24.5, P<.001	H=21.8, P<.001	H=21.6, P.001	H=24.9, P<.001	

Tab. 2 Evaluation of open tubules after cleansing. Comparison of different cleansing strategies and sealers. Differences: Mann-Whitney scores

1. There was no difference on using EDTA or not.

Some studies have suggested that ultrasonic device is effective in removing the smear layer both from the tubules and from the dentinal surface along the entire post-space (32-34), but in deeper areas of post-space is more difficult to obtain a clean dentinal surface. Often the ultrasonic device is combined with the EDTA and/ or NaOCl. Some studies reported that the use of EDTA combined with sodium hypochlorite (35, 36), has been shown to cleanse the canal walls of the post-space, but the NaOCl causes the release of oxygen which can inhibit the polymerization of resinous materials (37, 38). Therefore the acid etching of dentin is still an essential step to obtain clean canal walls and a greater adhesion force of the restorative material, bond strength which can be further obtained with the use of an ultrasonic tool in combination with an irrigant solution of EDTA at 17% for its activation of during the cleansing of the post-space, as shown in this study.

In SubGroup A and B EDTA was not used, while in SubGroup C it was used; the recorded scores among SubGroups were statistically significant different and consequently the first null hypothesis, that there was no difference on using or not EDTA, was not accepted.

2. There was no difference on using the ultrasonic

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device or not.

During the irrigation the ultrasound action is due to the capability of making minimal caving, minimal and just limited to the tip of the instrument, the acoustic streaming instead appears to be more significant. The ultrasounds, when near the liquid molecules, creates positive and negative pressure bubbles, which became not stable, will collapse and create an implosion similar to a decompression vacuum; the explosion and implosion of the bubble will release impact energy with a cleansing effect, has been shown that the sodium hypoclorite which has been previously activated with ultrasound cleans more efficiently the root canal space because there will be an increase in the flow of the irrigant furthermore improving the antibacterial proprieties, washing inorganic and organic debris removal power; instead as regard to the EDTA activity the ultrasounds seem to be less effective, but in any case, will allow a better smear layer removal (39). In this study the EDTA in combination with the ultrasounds was the most effective in SubGroups B and C, probably because the positive and negative liquid pressure might be effective to remove the remained endodontic sealer layer. Therefore, the second null hypothesis of this study, that there was no difference



on using ultrasound device or not, was not accepted. However, in Groups 2-4 also the latest described procedure (Subgroup 3) could not completely clean the dentinal walls and open the tubules' oreficies. Also the opened tubules that were observed, appeared to be close immediately after its beginning, probably for the presence of the hydraulic endodontic sealer that at the time of the endodontic obturation flew into the tubules. This speculation makes the question if the presence of hydraulic endodontic sealer into the tubules can't permit the infiltration of the adhesive system and the creation of resin tags, perhaps reducing the bond strength to root dentin. The presence of visible piece of endodontic sealer/gutta-percha found in SubGroups A and B might be related with the stiffness of hydraulic endodontic sealer can reach after being completely set. If these speculations can be true, it should be explored the possibility to make the endodontic obturation without touching the coronal third of the root canal with the endodontic sealer to prevent possible interference with bonding steps. These aspects are still to be investigated.

## **CONCLUSIONS**

When hydraulic endodontic sealers are used, the root canal walls can be cleaned with more difficulty than traditional endodontic sealer.

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For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, DP and MM.; methodology, DP, MF AI, SG; software, EFC.; validation, MM., SG. and DP.; formal analysis, MM.; investigation, MM, DP, MF; resources, MM; data curation, MF, DP; writing—original draft preparation, DP, MM; writing review and editing, MM, DP, SG; visualization, AI; supervision, MF, DP.; project administration, MM; funding acquisition, none. All authors have read and agreed to the published version of the manuscript."

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## **Conflicts of Interest**

The authors declare no conflict of interest.

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