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ORIGINAL ARTICLE

The influence of smear layer in lateral channels filling

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Abstract This in vitro study evaluated the ability of a warm gutta-percha obturation system Thermafil to fill lateral channels in presence/absence of smear layer. Forty single-rooted extracted human teeth were randomly divided into two groups for which different irrigation regimens were used: group A, 5 ml of 5% NaOCl + 2.5 ml of 3.6% H_2O_2; group B, 5 ml of 5% NaOCl 5% + 2.5 ml of 17% ethylenediamine tetraacetic acid. A conventional crown-down preparation technique was employed. Obturation was performed using epoxy resin-based cement (AH Plus) and a warm gutta-percha plastic carrier system (Thermafil). Specimens were cleared in methyl salicylate and analyzed under a stereomicroscope to evaluate the number, length, and diameter of lateral channels. Lateral channels were identified in both groups at medium and apical thirds. Additional samples were prepared for scanning electron microscopy inspection to confirm the presence of smear layer in group A, and the absence of smear layer in group B. All lateral channels resulted filled in both groups. No statistically significant differences regarding number, length, and diameter were observed between the two groups. Smear layer did not prevent the sealing of lateral channels.

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Introduction

The anatomical complexity of the endodontic canal system limits the likelihood of achieving a complete filling after chemo-mechanical treatment. Besides the infection of dentinal tubules [17], the presence of lateral channels represents a potential clinical issue related to the presence of bacteria colonizing their lumen [30, 31]. Harbored by pulpal or inorganic debris, many bacteria may reside in these areas and concur with pathological conditions. These areas may be repopulated by other bacteria, such as *Enterococcus faecalis*, and may be the principal reason for a secondary endodontic disease or refractory infection [13, 28, 30, 31]. Endodontic sealers and gutta-percha should close and fill lateral channels and dentinal tubules to prevent bacterial growth and percolation of bacteria and their by-products through the apex [5, 25, 33].

Among the various systems proposed to fill and seal endodontic channels after instrumentation, Thermafil represents a relatively straightforward and standardizable clinical choice [2, 4, 12, 26, 33].

A recent study [31] proposed a novel clearing technique which allows the adaptation of gutta-percha and sealer to endodontic dentinal walls to be evaluated and both filled and unfilled lateral channels to be observed. Type of sealer may influence the outcome of the injection of lateral channels [31] and its distribution in root canals [34].

Alone or in association with H_2O_2, NaOCl is frequently used as irrigant solution because of its well-known proteolytic activity [1] and its antimicrobial action [18]. Unfortunately, it is not able to remove smear layer [1].

Endodontic instrumentation creates a smear layer on the root canal walls that occludes dentinal tubules and may protect microorganism from the effects of NaOCl irrigation [5, 8]. Moreover, smear layer could support the growth of entrapped bacteria predisposing periapical reactions [5].

The influence of smear layer in the filling ability of a sealing technique is not fully clarified, as its presence might represent, at least in theory, an obstacle to the penetration of sealer and gutta-percha inside lateral channels [21, 29].

Several authors have suggested that ethylenediaminetetraacetic acid (EDTA) solutions or EDTA-based lubricants acting on the inorganic residue may contribute to removing smear layer [10, 16], and these are frequently used in endodontic therapy.

The aim of this study was to evaluate the extent to which smear layer prevents the filling of lateral channel with gutta-percha and sealer. The test null hypothesis was that the presence of smear layer impaired the filling of lateral channels.

Materials and methods

Sample preparation

Forty non-carious extracted single-root, single canal human teeth were used in this study.

All the teeth included in the study were necrotic, as confirmed radiographically by the presence of periapical lesions.

Teeth were equally divided in two groups with a homogeneous distribution regarding tooth type and apical diameter. Roots with resorption, fractures, or open apices were preventively discarded. The root canals selected for the study had an initial apical diameter of 0.20 to 0.30 mm.

Apical foramina were directly measured on the tooth using an optical microscope (Kaps SOM 62 standard, Karl Kaps GMBH åt KG, Asslar, Germany).

Calculus or debris on the root surface were removed before endodontic treatment using number 7/8 Gracey curettes (Hu-Friedy, Chicago, IL, USA). All specimens were prepared by the same clinician.

The crown of each tooth was removed using a tapered diamond bur (#845.314.012 Komet Brasseler, Lemgo, Germany) mounted on a contra-angle high-speed hand piece (Ceramic, Castellini, Bologna, Italy).

Root instrumentation was performed using conventional crown-down technique followed by a step-back technique. Stainless steel K-files (F.K.G. Dentaire, La Chaux-de-Fonds, Switzerland) and Gates Glidden drills (Dentsply-Maillefer, Baillagues, Switzerland) were used. The apical instrumentation was performed to obtain a final apical diameter of 0.30 or 0.40 mm depending on tooth anatomy.

Two different irrigation regimens were used in two designed experimental groups: group A, (n=20 roots) 5 ml of 5% NaOCl solution (Niclor-5, Ogna, Milan, Italy) followed by 2.5 ml of 3.6% hydrogen peroxide solution (Ogna, Milan, Italy); group B (n=20 roots) 5 ml of 5% NaOCl solution (Niclor-5, Ogna) followed by 2.5 ml of 17% EDTA (Ogna, Milan, Italy).

Irrigation was repeated after the use of each instrument and NaOCl solution operated for 20 min.

After canal preparation, a final 1 ml aliquot of 17% EDTA solution was left in situ for 2 min and replaced by 1 ml of 5% NaOCl for 3 min.

All irrigation procedures were delivered with a 25-gauge needle (Molteni, Firenze, Italy) inserted in the canal half-length.

Thermafil (Thermafil, Tulsa Dental, Tulsa, OK, USA) was used according to manufacturer instructions. The Thermafil carrier was selected according to the size of the gauging master apical file. A small amount of sealer (AH Plus, Dentsply DeTrey GmbH, Konstanz, Germany) was positioned inside each canal using a small K-file (no. 15). Immediately after the canal obturation, each sample was filled with Coltosol (Coltene, Switzerland) in the coronal aspect and immediately immersed in tap water for 24 h.

All teeth were then immersed for 14 days in a demineralizing solution composed of 9% formic acid, 8% hydrochloric acid, and 10% sodium citrate. The solution was changed every 3 days while specimens were kept under continuous agitation (using an agitator 722 by Asal srl, Milan, Italy) during the whole procedure. At the end of the demineralizing process, two specimens (a maxillary lateral incisor and a mandibular second bicuspid) revealed a longitudinal fracture and were discarded. The roots were then rinsed in running tap water for 2 h, immersed in 99% acetic acid overnight, rinsed again in distilled water, dehydrated in ascending ethanol from 25% to 100%, and finally cleared and stored in methyl salicylate (Sigma, St Louis, MO, USA).

Optical microscope was used with magnifications increasing from 5 to 40×, as performed in a previous study [31], and aided by the use of a micrometer to detect the number, the diameter, and the length of lateral channels. Diameter of lateral channels was measured at the opening along the wall of the root canal. Lateral channels were recorded, making a note of different filling within apical and middle third of the roots.

Statistical analysis

Data were analyzed by applying logistic regression analysis performed using STAT 7.0 (STATA, College Station, TX, USA).

Scanning electron microscopy preparation

Additional samples (n=12, six for each group) were prepared using conventional crown-down technique followed by a step-back technique as described above. No root canal filling was made so that the dentin surface could be observed after irrigation with NaOCl (n=6) or EDTA (n=6). Once prepared, each sample was immediately immersed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer solution before preparation for scanning electron microscopy (SEM) inspection. Each sample was then longitudinally fractured, dehydrated in graded concentration alcohol, dried in a critical point drier (E 3000; Polaron, West Sussex, UK), then gold-sputtered (Sputter Coater; SPI, Toronto, Canada) and observed under SEM (JEOL, JSM 5200, Tokyo, Japan). Two photomicrographs were obtained at a magnification of 2,000× at coronal, medium, and apical thirds.

Additional root samples were prepared according to the method described above (NaOCl: n=4; EDTA: n=4) then filled using the Thermafil system and immersed in tap water for 1 week at 37°C. Each sample was then transversally sectioned with a slow-speed diamond saw underwater to obtain three different root segments, approximately in the middle of each third. Each segment was conserved and fixed in 4% glutaraldehyde in 0.2 M sodium cacodylate buffer solution at 4°C, dehydrated in graded concentration alcohol, dried in a critical point drier (E 3000; Polaron, West Sussex, UK) then gold-sputtered (Sputter Coater; SPI, Toronto, Canada), and observed using SEM (JEOL, JSM 5200, Tokyo, Japan). Two photomicrographs were obtained at a magnification of 50×.

All images were saved digitally using specific software (SemAfore; JEOL) and scored in a double-blind manner by two trained operators.

Results

The clearing technique allows the observation of all the lateral channels (both filled and unfilled ones). The samples observed showed no unfilled channels: All lateral channels were at least partially filled even only by the sealer. The percentage of unfilled channels was nought in both groups despite the anatomical variation between the two groups.

Table 1 reports the number of lateral channels observed in the two groups according to the kind of teeth included in the study and to their locations in medium or apical third of both maxillary and mandibular teeth. A total of 130 lateral channels were identified along the roots, most of them being localized in the apical third. The presence of lateral channels was evenly distributed between maxillary and mandibular teeth.

Table 1 Number of lateral channels observed in the two experimental groups and distribution of observation according to their localization in the apical and medium thirds

	NaOCl + H_2O_2		NaOCl + EDTA		Total
	Apical third	Middle third	Apical third	Middle third	
Central maxillary incisors	9	6	2	5	22
Lateral maxillary incisors	0	0	1	14	15
Central mandibular incisors	8	0	15	2	25
Lateral mandibular incisors	0	0	4	1	5
Maxillary canines	8	9	4	0	21
Mandibular canines	1	0	0	0	1
Second maxillary premolars	1	9	0	0	10
First mandibular premolar	5	3	10	4	22
Second mandibular premolar	0	0	8	1	9
Total	32	27	44	27	130

Table 2 reports a descriptive statistic regarding the length (μm) and the diameter (μm) of lateral channels in the two groups at apical and medium thirds observed under optical microscope. Samples treated with NaOCl + EDTA compared to samples treated with NaOCl and H_2O_2 revealed a small increase (not statistically significant) in the number of lateral channels identified. Maximum, minimum, average,

Table 2 Length (μm) and diameter (μm) of lateral channels observed under optical microscope

Irrigation	NaOCl/H_20_2		NaOCl/EDTA	
Length	Apical third	Medium third	Apical third	Medium third
Mean	161.72 (±228.11)	757.37 (±1519.67)	253.86 (±336.47)	373.89 (±540.42)
Min–max	30–1,200	20–6,500	20–1,500	60–2,000
Diameter	Medium third	Apical third	Medium third	Apical third
Mean	135.53 (±223.05)	54.79 (±64.56)	81.93 (±85.13)	71.77 (±62.47)
Min–max	20–1,000	3–300	4–400	4–300

and SD value of the length and the diameter of lateral channels were reported.

Lateral channels of the apical thirds were shorter and thinner compared with lateral channels at the middle thirds ($P<0.01$).

Table 3 reports lateral channel diameter opening for the two groups of irrigants tested. An interesting result was that the diameter of the majority of the lateral channels (81 out of 130) was less than 50 μm. A great number of lateral channels, with larger diameter and longer length, was reported in the apical third of specimens treated with NaOCl + EDTA compared to NaOCl + H_2O_2, which revealed higher values for the same parameters in the middle third, but both the results were not statistically significant.

Stereomicroscope analysis

Stereomicroscopic images of cleared roots showing the filling of lateral channels at different distances from the apex both in presence and in absence of smear layer are shown in (Figs. 1 and 2).

Scanning electron microscopy analysis

Specific areas were observed at a magnification of 50×. SEM evaluations confirmed the presence of the plastic carrier and of gutta-percha (Fig. 3). Several limited gaps were observed along the sections, probably due to preparation artifacts. Sealer thickness was observed at the interface between gutta-percha and dentin.

After treatment with NaOCl, dentin samples were completely covered by smear layer and smear plugs. The morphology of dentin was similar at coronal, medium, and apical thirds. In several samples, apical thirds were partially covered by dentin debris (dimensions: 3–45 μ) and presented limited grooves with small area of predentin partially covered by debris.

Table 3 Distribution of the diameter of the opening (orifices) of lateral channel in the two experimental groups

	NaOCl + H_2O_2	NaOCl + EDTA
≤10 μ	10	5
11–20 μ	9	7
21–50 μ	19	41
51–100 μ	9	18
101–150 μ	6	5
151–200 μ	0	1
201–300 μ	5	3
301–400 μ	0	1
>400 μ	1	0
Total	59	71

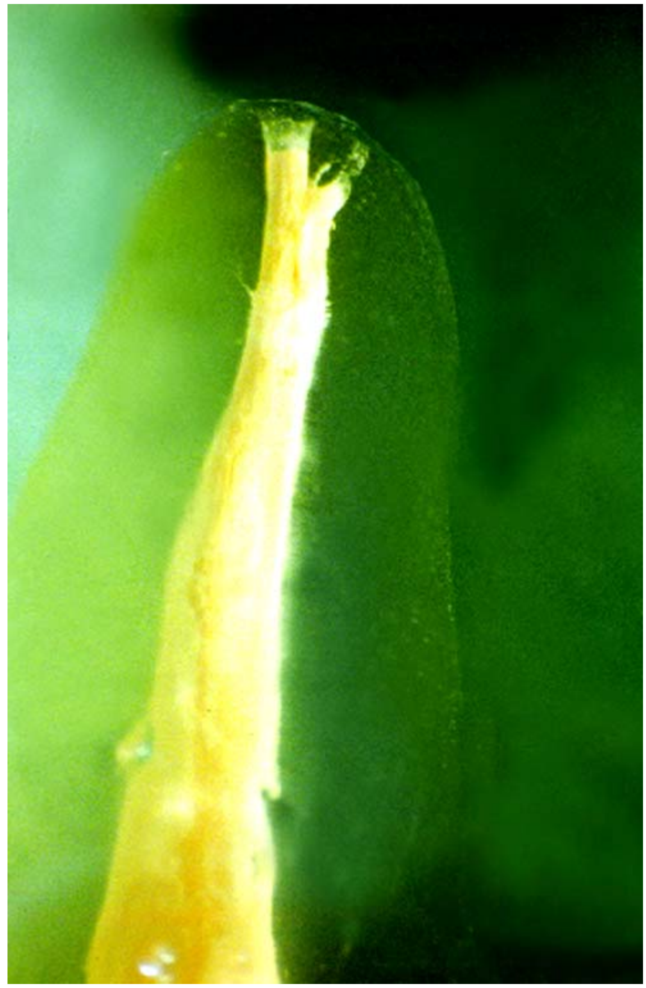

Fig. 1 Mandibular canine of the NaOCl–H_2O_2 group: the filling of apical bifurcation and lateral branches was detected

EDTA samples presented a smooth and smear layer free dentin with all dentinal tubules fully opened. Only apical thirds presented limited areas of compacted and partially layered smear layer islands.

Discussion

As no mechanical instrumentation can completely reach into all the root canal surface because of the complexity of the anatomy of the root canal system, the only clinical tools that can be used to reduce bacterial colonization are irrigants and filling materials [28, 30]. Rud and Andreasen [23] revealed that incomplete filling of lateral channels, as probably also the coronal leakage, may cause failure of endodontic treatment, as these empty areas represent pathways for bacteria and diffusion of toxins between endodontic and periodontal tissues. Moreover, endodontic failures that could be ascribed to incomplete sealing of lateral channels resulted in complete healing after filling of these areas [23].

Fig. 2 Second mandibular premolar of the NaOCl–EDTA group: apical branches of root canal were filled

Thus, the use of sealing materials able to penetrate and to fill lateral channels could be seen as the correct approach to prevent any further contamination and diffusion of bacteria present in the deepest part of dentinal tubules and lateral channels.

Previous studies indicated that filling techniques involving the use of thermoplasticized gutta-percha are very effective in filling the main root canal and lateral channels [9, 22]. Venturi et al. [31] described adequate filling of the lateral channels using a combined warm technique. In clinical use, Thermafil has been compared to cold lateral compaction technique when used with different endodontic sealers [3, 6, 9, 12, 26]. It still represents a simple and not clinician-sensitive method to fill root canals.

The clearing procedures used in this study allowed to be identified in both groups (NaOCl- and EDTA-treated samples) all lateral channels filled even only by the sealer. This data suggest that the insertion of Thermafil obturators toward the apical region may exert sufficient pressure to force endodontic sealer (AH Plus) and heated gutta-percha inside lateral channels. It is also plausible that smear layer and smear plugs may be pushed inside the lateral channels, especially in the NaOCl-treated root canals. The transparency induced by the clearing procedure could have been so good that it prevented the smear layer and smear plugs from being identified.

AH Plus was chosen for this study due to its low viscosity, which means it flows into thin spaces when used with warm gutta-percha obturation techniques [5, 13]. A previous study [31] found AH Plus to be a better sealer compared to other non-resinous endodontic sealers, agreeing with Haïkel et al. [11] who reported better performance of resin-based endodontic cements than non-resin-based ones.

Smear layer could be defined as a complex mixture of inorganic and organic particles constituting of dentinal collagen, pulpal debris, bacteria, and inorganic debris such as apatite [21, 27], created by all endodontic instruments [8]. Different endodontic procedures may produce a different amount of debris and a different morphology of smear layer [21] that may be greatly affected by design of instruments and methods of application and by the type of irrigation. Manual instrumentation with K-file produced a fine multi-layered smear layer. Sodium hypochlorite solution is able to remove pulpal debris and dentin collagen, but leaves smear layer intact [20, 29].

There has been a considerable debate about smear layer impact on endodontic treatment outcome [27, 29]. The question about presence/absence of smear layer is still controversial, and it is a problem of primary importance considering the possible role of smear layer in preventing lateral channels sealing, apical sealing, and bacterial contamination of dentinal tubules [21]. Previous studies demonstrated that it may harbor microorganisms and support their survival and growth [5]. Smear layer could also prevent or delay diffusion of irrigants and medicaments

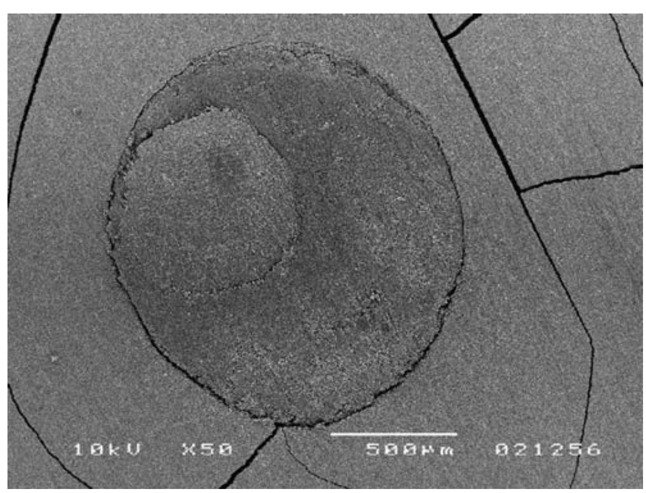

Fig. 3 Apical third section of a central maxillary incisor of the EDTA–NaOCl group with an off-center carrier, covered by a thin layer of gutta-percha, in absence of voids and gaps

into dentinal tubules and reduce the sealing ability of obturation materials [29]. On one hand, a detrimental effect of smear layer has been supposed by inducing bacteria contamination and preventing adequate adaption of sealers [21, 29]. On the other hand, smear plugs may be responsible for reduced permeability of root canal walls and for prevention of bacterial infiltration [21].

Two different irrigation regimens were selected and used in this study to evaluate the effective influence of smear layer in filling lateral channels. So, in group A, NaOCl and hydrogen peroxide were used as agents capable of leaving smear layer produced by K-files intact and unaltered, as confirmed by SEM analysis. In group B, in contrast, instead of hydrogen peroxide, EDTA was used to remove smear layer and smear plugs [19].

Several studies described the dentinal wall adaptation of thermoplasticized gutta-percha in absence or in presence of smear layer [5, 7]. In vitro studies demonstrated that removing smear layer significantly reduces apical leakage, which improves the seal (although other variables need to be considered, e.g., the kind of sealer) [5].

In contrast, other studies demonstrated that removing smear layer had no effect on the apical seal [7, 32] and no obliteration of accessory channels.

Furthermore, with regard to irrigation regimes and their correlation with enhanced penetration into the accessory channels, a recent study demonstrated that the removal of organic (NaOCl) and inorganic (EDTA) substance did not produce statistically significant differences in the obturation material penetration rate [32].

As suggested by the SEM pictures obtained during this study, the thickness of endodontic smear layer is probably 1–5 μ, and this smear layer must be easily pushed with enough pressure by warm gutta-percha inserted inside root channels. In spite of the use of a 17% EDTA solution, it is also plausible that in the apical area, debris and smear layer were also still present in the smear layer-free group. Recent studies demonstrated that the apical thirds also have a considerable amount of smear layer after using chelating agents [19]. The inability of EDTA to completely remove the smear layer from apical third may suggest that morphology of dentin in this area is similar in both groups.

For several reasons, it may be difficult for AH Plus and warm gutta-percha to penetrate smaller lateral channels partially or completely closed by smear layer. However, in this study, they appeared able to penetrate (or partially infiltrate) even smaller lateral channels in both groups.

Finally, a recent investigation by Saleh et al. [24] demonstrated that AH Plus adhesion to dentin may be negatively influenced by EDTA smear layer removal. They suggest that smear layer removal may impair sealer adhesion to dentin. These findings are extremely interesting, as they provide further insight into the results obtained in the study presented in this paper. A previous study of AH Plus observed greater penetration of lateral channels compared to other sealers [31]. Lee et al. [14] indicate that compared to other endodontic sealers, AH Plus has the highest bond strength to dentin (2.06 MPa) and also to gutta-percha (2.93 MPa). However, it is difficult to evaluate whether or not adhesive properties of sealer may improve penetration inside lateral channels and the interaction with smear layer.

The presence of a large number of open dentinal tubules at coronal and medium thirds in the EDTA-treated group may suggest that during the insertion of warm gutta-percha, many small gutta-plugs may penetrate the tubules and may reduce the pressure at the apical third. Furthermore, the thixotropic behavior of α-phase gutta-percha may have influenced the filling of lateral channels at the apical third [15]. Future studies should evaluate the penetration of gutta-percha and endodontic sealer inside dentinal tubules.

Conclusion

The goal of this study was to evaluate if removal of smear layer at the bottom orifice by EDTA improved the quality of lateral channels filling. The null hypothesis was rejected.

This study confirms that the presence of smear layer does not prevent the injection of lateral channels. In other words, the findings presented here support the concept that smear layer does not represent an obstacle to the penetration of sealers such as AH Plus and warm gutta-percha inside the lateral channels.

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