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Microscopic and chemical evaluation of the filling quality of five obturation techniques in oval-shaped root canals

Davide Mancino^{1,2} · Naji Kharouf^{1,2} · Mauro Cabiddu³ · Frédéric Bukiet^{4,5}  · Youssef Haïkel^{1,2}

Abstract

Objectives The aim of this study was to assess the filling quality of five obturation techniques in oval-shaped root canals.

Materials and methods A total of 212 mandibular first molars with one distal oval canal were selected. Distal canals, shaped with WaveOne Gold Primary, were randomly divided in five groups ($n = 40$) for obturation: continuous wave condensation, GuttaCore, Thermafil, single cone with AH plus, and single cone with BioRoot RCS. The proportions of gutta-percha-filled areas (GPFA), sealer-filled areas (SFA), void areas (VA), and the sealer/gutta tags into dentinal tubules at 4 mm and 2 mm from the apex were analyzed by an optical numeric microscope, SEM, and energy-dispersive X-ray (EDX). Data were then compared by Kruskal-Wallis one-way ANOVA on ranks ($\alpha = 0.05$).

Results At 4 mm, a statistically significant higher GPFA and lower SFA were observed in the GuttaCore and Thermafil groups compared with the 3 other groups. A statistically significant lower VA was observed in the continuous wave condensation, GuttaCore, and Thermafil groups than in the two single-cone groups. At 2 mm, there were a statistically significant higher GPFA and lower SFA and VA in GuttaCore and Thermafil groups than in the 3 other groups. At the two levels investigated, the presence of gutta-percha tags was clearly demonstrated for GuttaCore and Thermafil groups; no tags were observed in the 3 other groups. **Conclusions** Obturation quality was overall improved in GuttaCore and Thermafil groups.

Clinical relevance Carrier-based techniques may significantly improve the filling quality compared to continuous wave condensation and single-cone technique. The single-cone technique might have inherent limitations especially in oval root canals regardless of the sealer used.

Keywords Filling quality · Gutta-percha tags · Root canal · Void areas

Introduction

A successful endodontic treatment depends on appropriate access cavity preparation, shaping, cleaning/disinfection, and

tridimensional filling of the root canal space [1]. To date, there is no clear evidence to support any particular filling technique over another [2, 3] and the role of the obturation might be underestimated [4].

It is well known that cleaning/disinfection procedures do not eradicate 100% of bacteria in an infected root canal system [5]. Therefore, the obturation aims to entomb the residual bacteria and their by-products [6]. Many obturation techniques have been used. Voids incidence in root canal obturation has been reported especially in oval or irregular root canals [7]. This may result in proliferation of residual bacteria which may jeopardize the long-term outcome [8]. Since there is no adhesion between the gutta-percha and the dentin, the sealer aims to improve the sealability [9, 10]. However, an excessive sealer volume may negatively affect the seal, as zinc oxide eugenol or resin-based sealers tend to shrink upon setting and dissolve over time. Thus, the sealer-filled areas might be more vulnerable because of their dimensional instability [11, 12]. To counterbalance this issue, it has been

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recommended to maximize the gutta-percha volume and minimize the amount of sealer using thermoplasticized gutta-percha obturation techniques such as warm vertical compaction and carrier-based obturation [13]. More recently, hydraulic condensation has been proposed following the release of calcium silicate-based root canal sealers (CSBRCS). This technique does not require intracanal gutta-percha compaction but places strong emphasis on the improved properties of CSBRCS. However, a wide variation in the clinical use of CSBRCS has been highlighted [14]. Previous studies also reported a higher percentage of voids using CSBRCS rather than using epoxy resin-based sealer [15]. CSBRCS solubility has been highlighted to contribute to biologic properties whereas it may also negatively affect the quality of the sealing over time [11, 16, 17].

So far, there is a knowledge gap in comparing the filling quality of thermoplasticized gutta-percha obturation techniques and hydraulic condensation. Filling this gap is of prime importance considering that hydraulic condensation has gained in popularity in recent years.

The aim of this study was to assess the filling quality of five obturation techniques in oval-shaped root canals using optical numeric microscope analysis, SEM analysis, and EDX analysis to evaluate the percentage of gutta-percha-filled areas (GPFA), the sealer-filled areas (SFA), the void areas (VA), and the presence of gutta-percha/sealer tags. The first null hypothesis tested was that there is no difference in GPFA, SFA, and VA among the five groups tested. The second null hypothesis tested was that there is no difference in the presence of gutta-percha/sealer tags among the five groups tested.

Materials and methods

Samples

Four hundred and twelve freshly extracted mandibular first permanent molars with fully formed apices and with a total length between 21 and 23 mm were obtained, with patient informed consent. The protocol was approved by the Ethics Committee of Medical Odontology School, and Strasbourg University Hospital (protocol no. 2018-89). After debridement of the root surface, the specimens were immersed in a 1% sodium hypochlorite (NaOCl) solution at 4 °C for 24 h and then stored in saline solution.

Pre-selected teeth were scanned with CBCT and then selected according to the following morphological parameters:

1. One canal in the distal root.
2. A canal with a long/short diameter ratio \geq of 1.5 at 4 mm from the apex.
3. Canal with a total length of 10 ± 1 mm from canal orifice to apical foramen.

4. Primary root curvature in buccolingual and mesio-distal view $\leq 30^\circ$ according to the Schneider method [18].
5. Main curvature radius ≥ 4 mm.

After selection, two hundred and twelve teeth were included. The tooth length was standardized to 18 mm by removing the coronal portion of each tooth.

Sample size estimation was calculated with SigmaPlot release 11.2 (Systat Software, Inc., San Jose, CA, USA). Five groups of 40 teeth each were finally formed in order to have 95% power and an alpha error probability of 0.05.

Shaping procedure

A single experienced operator performed all clinical procedures. The specimens were mounted on protrain (Simit Dental SRL, Italy). After access cavity preparation, distal root canals were shaped using the following protocol. Scouting and glide path were established using a # 10 K file and working length (WL) was determined using the conductive protrain gel and an electronic apex locator (Root ZX; J Morita Co, Kyoto, Japan). Then, the apex was sealed with a cyanoacrylate glue in order to achieve a closed system. Before shaping the root canal, a pre-enlargement was performed using ProGlider (Dentsply Sirona) with an endodontic engine (X-Smart-IQ motor, Dentsply Sirona) at 300 rpm and 4 Ncm at full WL. Each canal was shaped with WaveOne Gold Primary (Dentsply Sirona) using the WaveOne mode of X-Smart-IQ motor.

After every two pecking motions, the reciprocating file was removed from the canal and the flutes cleaned. The canals were then irrigated with 1 mL of 6% NaOCl for (35 ± 5) s, for a total of 3 mL of NaOCl per canal, using a 5-mL syringe (Coltene/Whaledent, Altstätten, Switzerland) with 31-gauge Navitip needles (Ultradent Products, South Jordan, UT, USA) and the apical patency rechecked. This sequence was recapitulated until reaching WL with WaveOne Gold Primary.

Cleaning procedure

Each canal was dried using sterile paper points and was irrigated with 5 mL of 17% EDTA solution, 2 mL of physiological saline, 5 mL of a 6% NaOCl, followed by a final rinse with 2 mL of physiological saline. Each irrigant was agitated using endoactivator (Dentsply Sirona) for 180 s. Finally, each canal was dried with WaveOne Gold Primary-matched paper points.

Filling procedures

The teeth were randomly divided in five equal groups ($n = 40$ per group) for root canal obturation:

- Continuous Wave Condensation with AH plus (Dentsply Sirona)
- GuttaCore (Dentsply Sirona) with AH plus
- Thermafil (Dentsply Sirona) with AH plus
- Single-cone technique with AH plus
- Single-cone technique with BioRoot RCS (Septodont)

The remaining 12 molars were used as control teeth, using SEM analysis at 4 and 2 mm from the WL:

- 6 teeth after shaping procedure to observe the smear layer formation;
- 6 teeth after final irrigation protocol, to verify smear layer removal.

Continuous wave of condensation

A WaveOne Gold Conform Fit gutta-percha cone (WOGPC) was used to fill each canal. WOGPC was coated with a standardized amount of sealer (0.230 ± 0.005 g) and was gently inserted to WL. WOGPC was then down-packed to 5 mm from WL with a heated plugger, at 200 °C, and backfilled with warmed, cartridge-dispensed gutta-percha (Elements™ Free, Kerr Endodontics).

GuttaCore pink obturator

Each canal was filled with a GuttaCore obturator designed for WaveOne gold Primary. The same amount of AH Plus sealer was used to coat a sterile paper point to place the sealer into the root canal. The silicone stopper of the obturator was adjusted at WL. Then, the obturator was heated in a GuttaCore Oven (DentsplySirona) and was slowly and directly inserted to WL.

Thermafil system group

Each canal was filled with a Thermafil obturator designed for WaveOne gold Primary.

The same procedure used to fill the GC group was applied, with only one difference; the silicone stopper was placed 0.5 mm shorter from WL.

Single-cone technique with AH plus

A WOGPC was used to fill each canal of this group. The WOGPC was used to coat the canal walls with 2/3 of the standardized drop of AH plus sealer (0.230 ± 0.005 g). It was then recoated using the remaining amount of sealer and was slowly and directly inserted into the root canal up to WL. The cone was sealed off at the level of the canal orifice and slightly condensed with a plugger.

Single-cone technique with BioRoot RCS

The same procedure used to fill the previous group was applied except the choice of the sealer which was BioRoot RCS mixed according to manufacturer's instructions.

Sectioning and surface measurements

The specimens were stored in water at 37 °C for 72 h. After the storage period, a single experienced operator in optical microscopic, SEM, and chemical analysis, other than the one performing the clinical steps, carried out the microscopic experiment and its evaluation. After embedding the specimens in Epon 812 resin (Euromedex, Souffelweyersheim, France), two sections were cut perpendicular to the longitudinal axis of the root tooth at 2 mm and 4 mm from the apex with a wire saw (Walter EBNER, Le Locle, Switzerland). Thereafter, 1200, 2400, and 4000 P-grade (number of abrasive grains per cm²) abrasive paper was used, with continuous water irrigation. The polished samples were thoroughly rinsed with distilled water.

Optical numeric microscope analysis

The samples were first observed with an optical numeric microscope (KEYENCE, Osaka, Japan) then analyzed by using the VHX-5000 Communication software to measure the quantity of GPFA, SFA, VA.

SEM analysis

The specimens were dehydrated in a graded series of ethanol solutions and sputter-coated with a gold-palladium alloy (20/80 weight %) by using a HUMMER JR sputtering device (Technics, CA, USA). The coated samples were observed with a Quanta 250 FEG scanning electron microscope (FEI Company, Eindhoven, Netherlands) operating with an accelerating voltage of the electrons of 10 kV.

EDX analysis

The specimens underwent also a chemical analysis to check the GuttaCore Obturator, Thermafil obturator, WOGPC, AH plus sealer, root dentin, BioRoot RCS, and the related tags. Energy-dispersive X-ray (EDX) microanalysis was performed with an EDAX Octane Plus spectrometer (AMETEK Materials Analysis Division, 91 McKee Dr., Mahwah, NJ 07430, USA).

Statistical analysis

The collected data were analyzed with SigmaPlot release 11.2 (Systat Software, Inc., San Jose, CA, USA). Shapiro-Wilk test

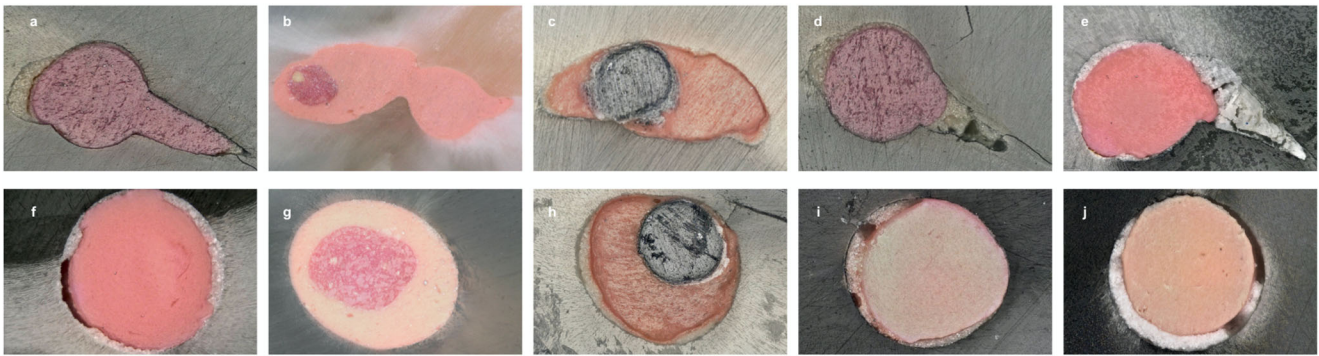


Fig. 1 Representative photos of a numeric optical microscope of sectioned root surfaces at 4 mm from the WL (a–e) and 2 mm from the working length (f–j). a, f Continuous Wave Condensation; b, g GuttaCore; c, h Thermafil; d, i single cone with AH plus; e, j single cone with Bioroot RCS

was used to verify the normality of data. Because the normality was not verified, the proportions of GPFA, VA, and SFA, corresponding to each filling technique, were compared by Kruskal-Wallis test (one-way analysis of variance on ranks) including a multiple comparison procedure (Tukey test), with statistical significance set at $\alpha = 0.05$.

Results

Optical microscope observations

For each group GPFA, SFA and VA were observed and measured at WL-4 mm and WL-2 mm (Fig. 1). All measurements were expressed in mean \pm standard error on the mean ($n = 40$) (Tables 1 and 2). At WL-4 mm, GuttaCore and Thermafil groups produced significantly ($\alpha < 0.05$) higher GPFA and lower SFA than the other groups, while no statistical difference ($\alpha > 0.05$) occurred between GuttaCore and Thermafil groups. No statistical difference ($\alpha > 0.05$) was detected among Continuous Wave Condensation, GuttaCore, and Thermafil groups when VA was analyzed. These filling techniques produced significantly ($\alpha < 0.05$) lower VA than the single-cone techniques.

Table 1 Comparison of GPFA, sealer area, and void area between the five obturation techniques in the samples at 4 mm from working length. Superscript letters a, b, c, and d indicate statistical significance ($P < 0.05$)

Groups	(n)	GPFA (%)	Sealer area (%)	Voids (%)
CWC	40	90.37 \pm 3.72 ^a	9.58 \pm 3.72 ^c	0.05 \pm 0.05 ^a
GC	40	99.43 \pm 0.44 ^a	0.51 \pm 0.43 ^{a, d}	0.05 \pm 0.04 ^a
TH	40	99.49 \pm 0.23 ^a	0.45 \pm 0.23 ^{a, d}	0.06 \pm 0.05 ^a
SC1	40	61.37 \pm 11.39 ^b	33.44 \pm 10.16 ^{b, d}	5.19 \pm 3.28 ^b
SC2	40	64.17 \pm 7.77 ^b	31.55 \pm 6.31 ^{b, d}	4.28 \pm 2.25 ^b
P value < 0.05		a–b	a–b, c–d	a–b

At WL-2 mm, GuttaCore and Thermafil groups produced significantly ($\alpha < 0.05$) higher GPFA and lower SFA and VA than Continuous Wave Condensation and the two single-cone groups ($\alpha = 0.05$). For the three parameters analyzed, no statistical difference ($\alpha > 0.05$) was detected between GuttaCore and Thermafil groups, as well as among Continuous Wave Condensation, single-cone with AH plus, and single-cone with BioRoot RCS.

With regard to the six control teeth examined after shaping procedure, all showed a smear layer covering 100% of dentinal tubules at the two levels investigated. However, the six teeth examined after the cleaning procedure showed, respectively, at WL-4 mm and at WL-2 mm, 72% and 53% of open dentinal tubules.

Scanning electron microscope results

SEM analyses assessed the presence of intratubular tags. No tags were observed at WL-4 mm and WL-2 mm in the Continuous Wave Condensation specimens, as well as in the two single-cone technique specimens, whereas in each GuttaCore and Thermafil specimens, the presence of tags was clearly demonstrated (Fig. 2).

Table 2 Comparison of GPFA, sealer area, and void area between the five obturation techniques in the samples at 2 mm from working length. Superscript letters a and b indicate statistical significance ($P < 0.05$)

Groups	(n)	GPFA (%)	Sealer area (%)	Voids (%)
CWC	40	77.69 \pm 3.88 ^a	19.34 \pm 3.52 ^a	2.97 \pm 0.90 ^a
GC	40	99.25 \pm 0.42 ^b	0.64 \pm 0.40 ^b	0.11 \pm 0.06 ^b
TH	40	99.26 \pm 0.49 ^b	0.62 \pm 0.48 ^b	0.12 \pm 0.10 ^b
SC1	40	78.05 \pm 3.74 ^a	19.13 \pm 3.52 ^a	2.82 \pm 0.95 ^a
SC2	40	77.03 \pm 2.02 ^a	19.99 \pm 1.83 ^a	2.98 \pm 0.85 ^a
P value < 0.05		a–b	a–b	a–b

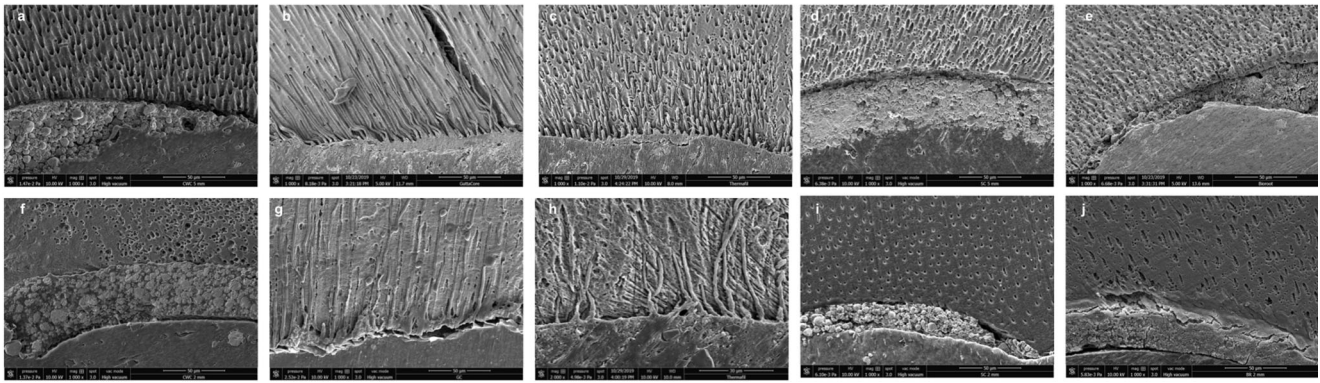


Fig. 2 Representative photos of scanning electron microscopy ($\times 1000$) of sectioned root surfaces at 4-mm level from the working length (a–e) and 2 mm level from the working length (f–j). a, f Continuous Wave

Condensation; b, g GuttaCore; c, h Therafil; d, i single cone with AH plus; e, j single cone with BioRoot RCS

Chemical analysis findings

The spectrums of WOGPC, GuttaCore obturator, Therafil obturator, AH plus sealer, BioRoot RCS, and root dentin were likened to the spectrum of GuttaCore tags and of Therafil tags (Figs. 3 and 4). This allowed to state that the GuttaCore and Therafil tags, at WL-4 mm and at WL-2 mm, consist of gutta-percha.

Discussion

The present study aimed at assessing the filling quality of five obturation techniques in oval-shaped root canals based on GPFA, SFA, VA evaluation, and gutta-percha/sealer tag observation.

Unlike earlier studies [13, 19–21], CBCT use for sample selection reduced the lack of homogeneity among dental specimens. The latter were carefully polished providing high reflective surface limiting potential artifacts [13].

In this study, the filling ability of different techniques was evaluated at 2 and 4 mm from the WL using an optical digital microscope, in order to evaluate GPFA, sealer, and void area percentages and interfacial gaps in apical and middle parts of the root canal.

Previous studies used an optical or a SEM microscope at magnifications ranging from 50 up to $\times 200$ [13, 20]. In the present study, the samples were at first observed, before dehydration, using an optical numeric microscope with composition pictures taken at magnification of $\times 500$, in order to maintain the original resolution. Then, they were analyzed to measure GPFA, SFA, and VA. It has been showed that samples' dehydration and examination in a high vacuum environment can lead to artefactual interfacial gaps [22]. This was avoided by limiting the use of SEM to highlight the intratubular penetration of filling materials (Fig. 5).

Other studies used the confocal laser scanning microscopy to detect intratubular penetration of filling materials. Nevertheless, the autofluorescence of dentin, when exposed to a wavelength of 405 to 600 nm, should contraindicate its use without the FLIM technique [23]. However, to the best of

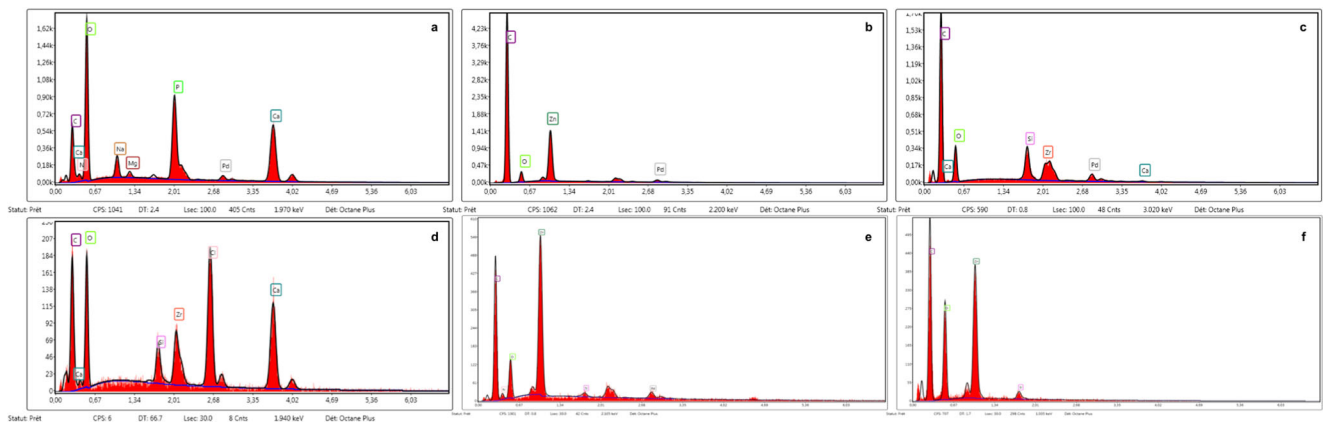


Fig. 3 Energy-dispersive X-ray spectrum of (a) dentine, (b) gutta-percha cone, (c) AH Plus sealer, (d) BioRoot sealer, (e) GuttaCore gutta-percha, and (f) Therafil gutta-percha. The palladium peak comes from the metal coating for SEM

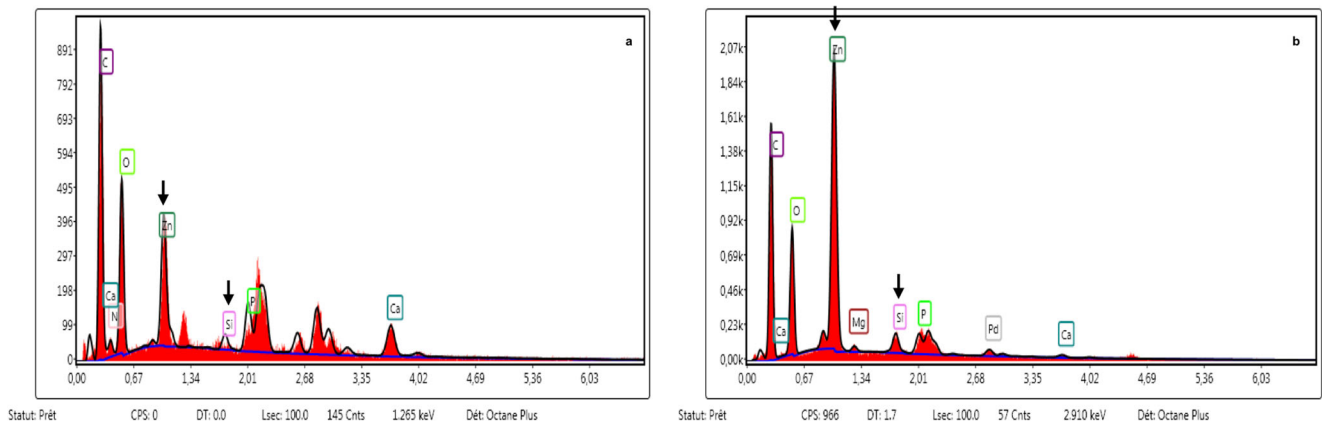


Fig. 4 Energy-dispersive X-ray spectrum of (a) Thermafil tag specimen and (b) GuttaCore tag specimen

our knowledge, previous investigations showing the presence of intratubular tags at the level investigated in the present study did not use the FLIM technique questioning the interpretation of their results [24, 25]. Moreover, there is no clear evidence that fluorescein and rhodamine chemically link to the different sealers also questioning this methodology. The use of micro-CT which is a non-destructive method grows in popularity to evaluate endodontic procedures including root canal filling quality. Like sectioning and microscopic observation, this technique remains time consuming. It also requires a thorough computer expertise. Moreover, micro-CT usage can lead to artifacts in the reconstructed images such as beam-hardening complicating the interpretation of the image [26, 27] especially images of unmineralized structures [28]. Thus, this destructive investigation method using a numerical optical microscope with composition images taken at a magnification of $\times 500$ (Fig. 6) could be a reliable alternative to the sub-optimal micro-CT axial sections resolution [21].

In the present study, at WL-4 mm, GuttaCore and Thermafil groups produced significantly higher GPFA and lower SFA than the 3 other groups. Nevertheless, no significant statistical difference was found between GuttaCore and

Thermafil groups, as well as among the 3 other groups. No statistical difference was detected among Continuous Wave Condensation, GuttaCore, and Thermafil groups when VA was analyzed. However, lower VA were observed using thermoplasticized gutta-percha obturation techniques than in the single-cone groups. In the present study, no device helping sealer injection and distribution within the root canal (such as Lentulo spiral or injection tip) was used. This may explain higher VA when using a single-cone technique in oval canals compared to warm gutta-percha obturation methods which combine hydraulic and mechanical condensation. If the clinical significance of voids can be debatable, the latter could theoretically shelter remaining bacteria and their by-products with negative impact on the clinical outcome.

The present results are in accordance with those of previous studies showing significantly higher GPFA and lower SFA for Thermafil group [19], no difference in VA between Continuous Wave Condensation, and GuttaCore groups [21] and no difference between GuttaCore and Thermafil groups for GPFA, SFA, and VA [29]. However, a previous study found no difference, between Continuous Wave Condensation and Thermafil groups at WL-5 mm in terms

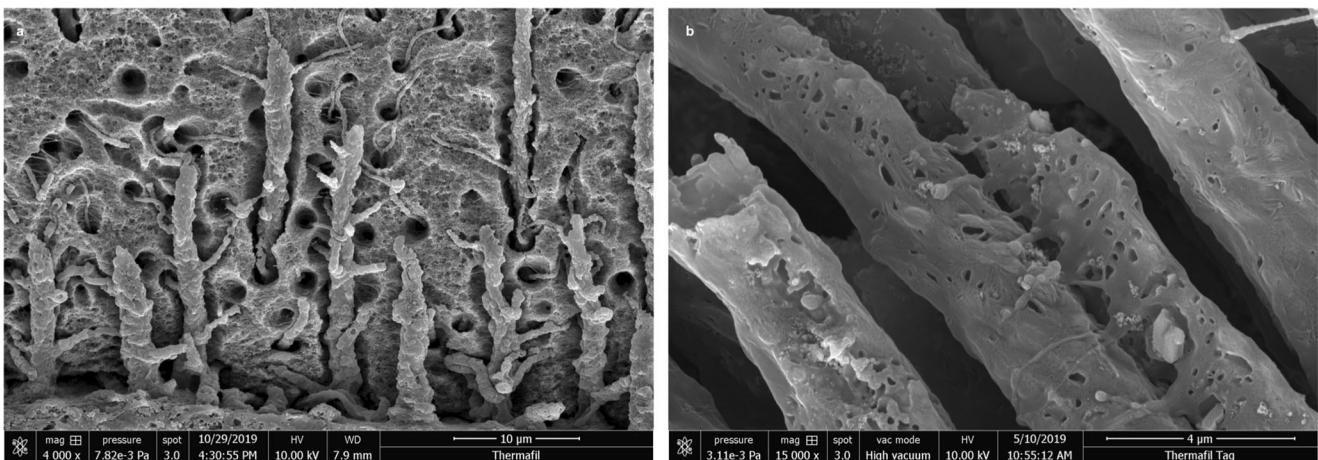
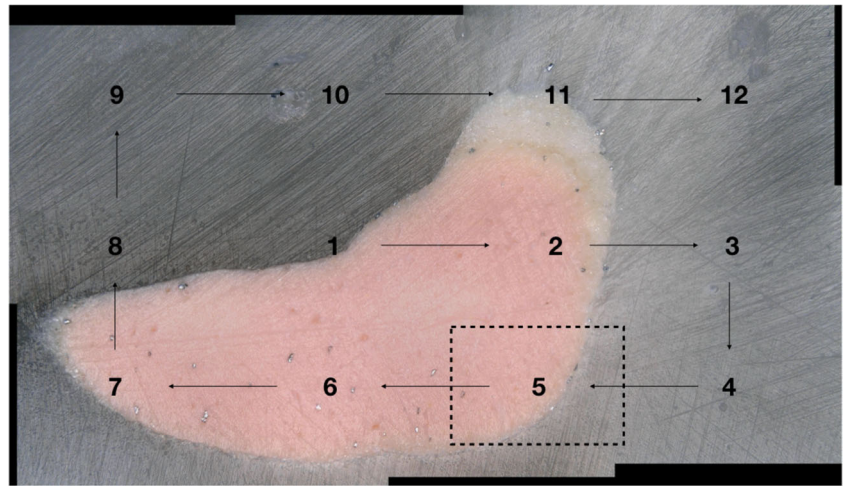


Fig. 5 a, b Gutta-percha tags at different magnifications

Fig. 6 Representative photo taken with a numerical optical microscope using composition image technology. Notice that every single composition image has a magnification of $\times 500$. At 4 mm from the WL, each photo was composed of no less than 12 composition images



of GPFA and VA [30]. However, this investigation did not mention the irrigation protocol and no sealer was used [30].

In the present study, Continuous Wave Condensation group showed statistically higher SFA than GuttaCore and Thermafil groups. This may be due to the heat plugger use which may increase AH plus thickness [31]. No statistically significant difference was observed when considering GPFA, SFA, and VA between the single-cone techniques, suggesting that this technique might have inherent limitations in oval root canals regardless of the type of sealer used.

At WL-2 mm, the GuttaCore and Thermafil groups, showing plasticized gutta-percha, produced significantly higher GPFA, lower SFA, and lower VA than the three other groups. There was no statistical difference among Continuous Wave Condensation and the two single-cone techniques in terms of GPFA, SFA, and VA. No specimen of Continuous Wave Condensation showed plasticized gutta-percha at WL-2 mm confirming that this technique behaves as a single-cone technique in the apical third the gutta-percha being a poor thermal conductor [32, 33].

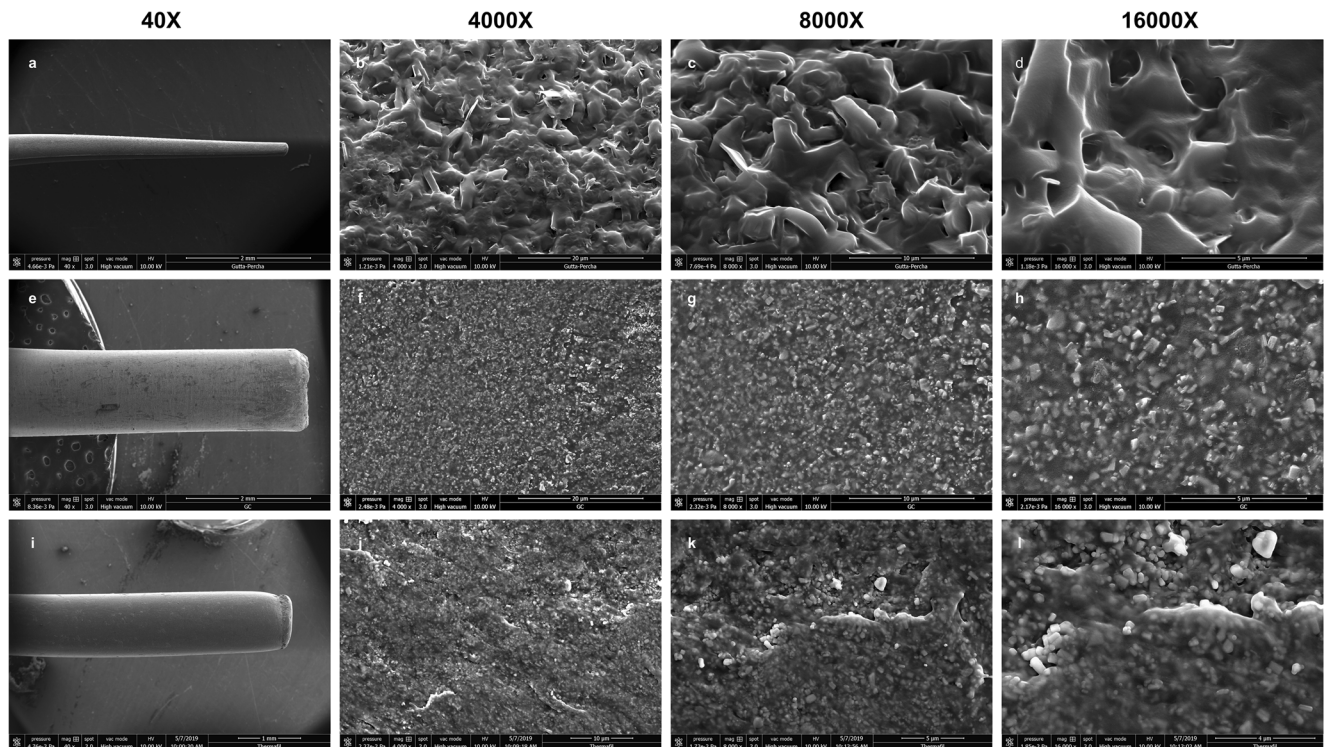


Fig. 7 a–d Scanning electron microscopy image of gutta-percha cone, at different magnifications, reveals a large size of the different constituents. e–h Scanning electron microscopy image of GuttaCore gutta-percha, at different magnifications, reveals a smaller size of the different

constituents. i–l Scanning electron microscopy image of Thermafil gutta-percha, at different magnifications, reveals a smaller size of the different constituents

The present results are in accordance with those of previous studies showing that in the apical third, significantly lower VA was observed for Thermafil group compared to single-cone group [34], no difference in VA was observed among Continuous Wave Condensation technique, single-cone technique using epoxy resin-based sealer and single-cone technique using calcium silicate-based sealer [25], and no difference occurred between GuttaCore and Thermafil groups for GPFA, SFA, and VA [29].

In the present study, SEM observation disclosed intratubular tags in GuttaCore and Thermafil groups at WL-4 mm and WL-2 mm. EDX analyses demonstrated that the tags were composed of gutta-percha as described in a previous study [29]. No sealer tags were observed in the present study. No specimen of Continuous Wave Condensation group showed gutta-percha tags at WL-4 mm and WL-2 mm. Since the gutta-percha is not plasticized in the apical third, the absence of gutta-percha tags at WL-2 mm is obvious. The absence of gutta-percha tags at WL-4 mm is explained by the size of the gutta-percha crystals bigger than those of GuttaCore and Thermafil obturators and bigger than the diameter of the dentinal tubules (Fig. 7). Moreover, the low viscosity of Thermafil [35] and GuttaCore gutta-percha might also contribute to the presence of gutta-percha tags in these groups.

The results of the present study permit to reject the first and second null hypothesis.

Conclusions

Within the limitations of this study, carrier-based obturation techniques might be considered appropriate to fill oval-shaped root canals. In the apical third, single-cone techniques and Continuous Wave Condensation technique showed different limitations regardless of the sealer used. Finally, further investigations are necessary to better understand the clinical implications of intratubular penetration of filling materials.

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Compliance with ethical standards

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

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the Ethics Committee of Medical Odontology School, and Strasbourg University Hospital (protocol no. 2018-89) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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