Percentage of Gutta-Percha-, Sealer-, and Void-Filled Areas in Oval-Shaped Root Canals Obturated with Different Filling Techniques: A Confocal Laser Scanning Microscopy Study

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Objective This study compared different obturation techniques, analyzing percentage of areas filled with gutta-percha, sealer, and voids (PGFA, PSFA, and PVFA, respectively) in oval-shaped root canals.

Materials and Methods A total of 60 extracted human mandibular central incisors were decoronated, instrumented, and irrigated using the same protocol. After drying, the root canal was filled with AH Plus labeled with 0.1% rhodamine B dye using a Lentulo spiral. The filling procedure was performed by dividing the teeth into four groups according to the respective technique: G1, cold lateral condensation; G2, continuous wave of condensation; G3, modified cold lateral condensation using an F3 master cone; and G4, modified continuous wave of condensation using an ISO (International Organization for Standardization) sized 30 gutta-percha cone. Then, slices measuring 1.5 mm in thickness were obtained 3 and 6 mm from the apex and evaluated by confocal laser scanning microscopy to determine PGFA, PSFA, and PVFA.

Statistical Analysis The data were analyzed statistically with analysis of variance and Games-Howell’s tests ($p = 0.05$).

Results The groups showed no significant differences in the apical third (3 mm from the apex). In the middle third (6 mm from the apex), G3 and G1 showed higher PGFA and PVFA, respectively. G3 showed lower PSFA than G2 and G4. Both cold techniques (G1 and G3) promoted lower PSFA than both warm techniques (G2 and G4).

Conclusions Notwithstanding the limitations of this in vitro study, PGFA, PSFA, and PVFA ranged significantly only in the middle third, as observed by the different filling techniques. Higher PGFA and PVFA values were obtained for G3 and G1, respectively. Both cold techniques promoted lower PSFA than both warm techniques.

Introduction Endodontic treatment aims to maintain or reestablish the health of periapical tissues by cleaning and filling the root canals.1,2 Biomechanical preparation and intracanal dressing (when used) are responsible for disinfection; however, they are not able to completely eliminate the root canal system content.3,4 Therefore, an effective root canal filling must be performed to maintain cleanliness, trap remaining...
microorganisms, interrupt the supply of nutrients needed for their survival, and avoid contamination or recontamination.\(^5\)

Obturation has most commonly been performed with gutta-percha and a sealer. Schilder\(^1\) and Epley et al\(^8\) suggested that root canal filling material should adapt analogically to the canal walls as well as its irregularities. Furthermore, it is important that gutta-percha be placed along the entire length of the canal, be densely compacted, and consist of a homogeneous mass. For this reason, a widely used strategy to evaluate the quality of obturations is to analyze the percentages of areas filled with gutta-percha, sealer and voids (PGFA, PSFA, and PVFA, respectively).\(^7,8\)

The cold lateral condensation technique (CLCT) is the most common approach used in endodontic clinics. Its advantages include relative ease of use, low cost, predictability, and controlled placement. However, this technique has been known to leave voids, use an excessive amount of sealer, and be deficient in adapting gutta-percha adequately to root canal walls.\(^5\)

The warm vertical condensation technique enables gutta-percha to be provided in a homogeneous and dimensionally stable mass; this makes it easier for the material to penetrate root canal system ramifications. This technique has been simplified by certain recently introduced devices such as System B (SybronEndo; Orange, California, United States). This or similar equipment allows the heating of the filling material in a single step. Moreover, this approach leads to the creation of the continuous wave of condensation technique (CWCT). CWCT is not only a more costly technique, requiring specific equipment, but it also promotes higher extrusion of filling materials.\(^5\)

No obturation technique can completely fill the root canal.\(^5\) This limitation is even more critical in teeth with oval canals, such as the mandibular incisors.\(^9,10\) For this reason, different materials,\(^1,3,9,11\) techniques,\(^1,3,14\) and modifications\(^15,16\) have been proposed and studied using several methodologies.\(^17,18\)

One of the preferred tools to assess the adhesive interface and the topographic features of the root canal filling is confocal laser scanning microscopy (CLSM). It stands out because it allows samples to be measured in depth despite a wet environment. A fluorescent dye is used to mark root canal sealers so that they can be analyzed under CLSM. Certain dyes are added to sealers to cause certain wavelengths to excite the marked structure for the purpose of making the spectrum visible. CLSM is capable of accurately determining the degree of adaptation and penetration of the root canal filling materials.\(^18\)

This in vitro study aimed at determining PGFA, PSFA, and PVFA in oval-shaped root canals obturated with CLCT and CWCT and assessing two respective procedural modifications using CLSM.

## Materials and Methods

### Tooth Selection

Sixty extracted human mandibular central incisors were selected for this study after approval by the University Research Ethics Committee (no. 5690/11). The selection was based on acquiring teeth with straight and single root canals ending in just one main apical foramen, without radicular cracks, resorption process, or other anatomical complexities, and with prior endodontic treatment. Buccal and proximal radiographs, and a stereomicroscope under 20× magnification (Expert DN; Mueller-Optronic, Erfurt, Germany) were used to confirm these features. The teeth were kept in a 0.1% thymol solution at 4°C until use.

### Root Canal Shaping and Filling

The roots of the teeth were standardized at 14 mm in length by sectioning tooth crowns with a low-speed steel cutting disc (IsoMet, Buehler, Lake Bluff, Illinois, United States). Root canal orifices were prepared with a 3082 tapered-tip bur (KG Sorensen, São Paulo, Brazil) and a Largo No. 2 drill (Dentsply-Maillefer, Ballaigues, Switzerland), whereas cervical and middle thirds were prepared using Gates-Glidden No. 3 and No. 2 drills (Dentsply-Maillefer). The working lengths (WLs) were established at 1 mm short of the point where a 15-K file (Dentsply-Maillefer) was visible at the apical foramen.

Instrumentation of root canals used the crown-down technique and rotary ProTaper instruments (Dentsply-Maillefer) up to size F3 at the WL. At each change of file, the canals were irrigated with 2.5 mL of 2.5% NaOCl (Fórmula & Ação, São Paulo, Brazil) prepared immediately before, together with a flush of 3 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Fórmula & Ação) for 3 minutes. The final rinse consisted of 5 mL of sterile water. The root canals were dried with sterile absorbent paper points (Tanari, São Paulo, Brazil). A 10-μL aliquot of AH Plus (Dentsply DeTrey, Konstanz, Germany), labeled with 0.1% Rhodamine B dye (Sigma-Aldrich, St. Louis, Missouri, United States), was placed into the root canal using a size 20 Lentulo spiral in a counterclockwise rotation.

The filling procedure was performed by dividing the teeth into four groups (n = 15) according to the respective technique (see Table 1). The teeth were then stored in 100% humidity at 37°C for 2 weeks.

### Confocal Laser Scanning Microscopy

The filled specimens were embedded in crystal resin to obtain 1.5-mm-thick cross-sections 3 and 6 mm from the apex using a diamond cutting disc (Ø 127 vmm × 0.4 vmm × 12.7 vmm, Buehler, Ltd. Lake Bluff, IL, United States) coupled to an automatic precision cutter (IsoMet 4000, Buehler Ltd.) at a speed of 5 mm/minute at 400 rpm rotational speed and under abundant water cooling, resulting in a total of 120 slices. The coronally facing surface of each slice was polished with a size 20 Lentulo spiral using a 127 vmm × 0.4 vmm × 12.7 vmm, Buehler, Ltd. Lake Bluff, IL, United States) coupled to an automatic precision cutter (IsoMet 4000, Buehler Ltd.) at a speed of 5 mm/minute at 400 rpm rotational speed and under abundant water cooling, resulting in a total of 120 slices. The coronally facing surface of each slice was polished with a size 20 Lentulo spiral in a counterclockwise rotation.

A fluorescent dye was used to mark root canal sealers so that they can be analyzed under CLSM. Certain dyes are added to sealers to cause certain wavelengths to excite the marked structure for the purpose of making the spectrum visible. CLSM is capable of accurately determining the degree of adaptation and penetration of the root canal filling materials.\(^18\)

Images were then taken by CLSM (Leica, Jena, Germany) for analysis of the PGFA, PSFA, and PVFA using two Ne-He laser beams excited with a 568-nm wavelength. The teeth were scanned axially and laterally using the fluorescence mode with a resolution of 220 and 330 nm, respectively. Root third images were obtained by Leica Microsystems LAS AF TCS MP5 software (Leica, Jena, Germany). The images were analyzed using a noncompressed format (.TIFF) (see Fig. 1), and the measurements were performed using Adobe Photoshop Cs3 (Adobe, San Jose, California, United States) by a single operator. Each section was measured three times, and the means were calculated. Areas of gutta-percha, sealer, and voids were determined using image analysis.
void were converted into percentages (PGFA, PSFA, and PVFA, respectively) of the total canal area.

Statistical Analysis
The data were analyzed statistically using analysis of variance and Games–Howell’s tests, with a significance level set at $p = 0.05$.

Results
No significant differences in PGFA, PSFA, and PVFA were observed in the apical thirds among the groups ($p > 0.05$). The higher PGFA and PVFA values were obtained in G3 and G1, respectively ($p < 0.05$). Both G1 and G3 promoted lower PSFA than G2 and G4 ($p < 0.05$) (Table 2).

Discussion
Obturation of a shaped and cleaned root canal is most effective when a maximum amount of gutta-percha is packed into the canal, and sealer amounts are kept to a minimum because most sealers shrink on setting and dissolve over time, unlike gutta-percha, which is dimensionally stable. For this reason, PGFA, PSFA, and PVFA were used in the investigation to assess obturation quality based on the procedure used in previous studies.

The main disadvantages of CLCT and CWCT are the excessive quantity of sealer and voids and the extrusion of filling materials, respectively. For these reasons, we proposed two respective procedural modifications to offset these adverse features, namely, the use of matched tapered master cones for performing CLCT (G3–MCLCT) and standardized cones for performing CWCT (G4–MCWCT).

In regard to PGFA, PSFA, and PVFA, no significant differences in the apical third (3 mm from the apex) were observed among the groups ($p > 0.05$), corroborating the results found in previous studies. Leoni et al. used microcomputed tomography (CT) to study the anatomy of 100 mandibular central and lateral incisors. At 3 mm from the apex, mandibular central and lateral incisors showed areas of $0.16 \pm 0.12$ and $0.14 \pm 0.08 \text{ mm}^2$ and roundness of $0.43 \pm 0.22$ and $0.46 \pm 0.21$, respectively. This study performed instrumentation up to the

Table 1  Groups and protocols for root canal fillings

<table>
<thead>
<tr>
<th>Group</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (CLCT)</td>
<td>Cold lateral condensation technique was performed with an ISO-sized 30 gutta-percha master cone (Tanari). A size 25 NiTi finger spreader calibrated up to 1 mm from the WL was introduced up to the allowed depth. R7 accessory cones (Tanari) were used for lateral condensation. As many accessory cones as possible were placed in the canal.</td>
</tr>
<tr>
<td>G2 (CWCT)</td>
<td>Continuous wave of condensation technique was performed using an F3 master cone (Dentsply-Maillefer) with a sectioned tip locked at 1 mm short of the WL. The heat carrier of System B (SybronEndo) was used up to 5 mm from the WL.</td>
</tr>
<tr>
<td>G3 (MCLCT)</td>
<td>The same protocol described for G1 but using an F3 master cone.</td>
</tr>
</tbody>
</table>

Abbreviations: CLCT, cold lateral condensation technique; CWCT, continuous wave of condensation technique; ISO, International Organization for Standardization; MCLCT, modified cold lateral condensation technique; MCWCT, modified continuous wave of condensation technique; WL, working length.

Fig. 1 Illustrative confocal laser scanning microscopy image used for analysis.
Table 2  PGFA, PSFA, and PVFA in each third

<table>
<thead>
<tr>
<th>Group (n = 15)</th>
<th>Apical third</th>
<th>Middle third</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PGFA, mean ± SD</td>
<td>PSFA, mean ± SD</td>
</tr>
<tr>
<td>G1</td>
<td>67.30 ± 11.76b</td>
<td>29.84 ± 11.33b</td>
</tr>
<tr>
<td>G2</td>
<td>62.77 ± 6.54b</td>
<td>36.59 ± 6.71b</td>
</tr>
<tr>
<td>G3</td>
<td>65.79 ± 12.20b</td>
<td>32.50 ± 11.89b</td>
</tr>
<tr>
<td>G4</td>
<td>56.60 ± 8.91b</td>
<td>42.22 ± 11.22b</td>
</tr>
</tbody>
</table>

Abbreviations: PGFA, percentage of areas filled with gutta-percha; PSFA, percentage of areas filled with sealer; PVFA, percentage of areas filled with voids.

Note: Different superscript letters indicate statistically significant differences (p < 0.05).

F3 file at the WL established at 1 mm short of the apical foramen. The canals were then instrumented up to approximately 0.48 mm² at 3 mm from this region. This shaping process was responsible for regularizing the canals in terms of circularity, mainly in the apical third, thus increasing obturation quality, regardless of the technique.²²,²³

In the middle third (6 mm from the apex), G1 presented higher PVFA than the other groups (p < 0.05). Several studies have shown limitations of the CLCT for filling oval-shaped canals in the cervical and middle thirds.¹⁰,²⁴ Schäfer et al.²⁵ evaluated PGFA, PSFA, and PVFA after shaping and filling 60 extracted mandibular incisors with the following systems: (A) FlexMaster (VDW Antaeos; Munich, Germany), (B) Mtwo (VDW; Munich, Germany), (C) ProTaper (Dentsply; Weybridge, Surrey, United Kingdom), (D) Reciproc (VDW; Munich, Germany), (E) WaveOne (Dentsply Maillefe; Ballaigues, Switzerland), and (F) manual instrumentation. In groups A–E and F, they used matching single-cone gutta-percha and CLCT, respectively, to perform the fillings. Curiously, group F produced similar PVFA results at the 6-mm level compared with group C. These results contrast with our findings, in which G1 presented higher PVFA than the other groups. However, it is noteworthy to mention an important point about the methodology used in the study performed by Schäfer et al.²⁵: there were four cases of teeth with an oval canal or a canal with isthmuses when the teeth were sectioned; in this case, they were replaced by a new one. The authors did not report from which groups these teeth were removed and replaced. This is an important bias of the study, which even may have influenced the results, considering that the performance of CLCT may be improved in teeth with circular canals.²²,²³

Still, in regard to the middle third, higher PGFA values were obtained in G3 compared with the other groups (p < 0.05). The accessory cone sizes and tapers chosen were smaller than the finger spreader so that GP could be placed in the entire extension of the space created.²⁶ However, in G3 specimens, finger spreader penetration was limited to the initial millimeters of the root canal because an F3 cone was used. In G1 specimens, the finger spreader reached the final millimeters of the root canal, mainly at the beginning of lateral condensation. The G1 specimens commonly showed large unfilled round-shaped voids (spreader tracks).²⁴ The reason the spreader tracks remained unfilled could be that there was a size variation in the accessory cones of the same brand²⁷ and that there was no standardization between spreader size and accessory cones.²⁸ The arguments mentioned above explain the higher PGFA and PVFA of both G3 and G1 compared with the other groups.

All thermoplasticized gutta-percha techniques have a common feature, which is the heating of gutta-percha to an extremely high temperature such as 200°C to 400°C to ensure that the gutta-percha mass melts homogeneously.²⁹ However, high-temperature settings risk degradation of gutta-percha and the formation of new compounds of low molecular mass such as peroxides and volatile products.³⁰ The loss of material stability and reduction in molar mass may explain both the higher PGFA of G3, compared with the other groups, and the lower PSFA of G3, compared with G2 and G4. According to Capurro et al.,³¹ 2 minutes after obturation using the warm vertical condensation technique, the gutta-percha underwent significant shrinkage. Moreover, there were large areas of no adaptation that increased even more after 5 minutes and 30 minutes.

This study used CLSM because it has advantages that are unavailable with scanning electron microscopy and histological methods. These include providing detailed information on gutta-percha, sealer, and void distribution in the total circumference of the root canal at a magnification as low as 50× to 100× using just one image.²²,²³ Furthermore, CLSM allows different analyses to be made under environmental conditions, with no special specimen processing, resulting in lower production of technique artifacts.³² The drawback is that CLSM does not give volume information, unlike micro-CT data, and uses a technique that can be considered as destructive. Therefore, it is important that future studies be conducted using micro-CT to elucidate the impact of each filling technique on the complex root canal anatomy.³³

Conclusion

Notwithstanding the limitations of this in vitro study, PGFA, PSFA, and PVFA ranged significantly only in the middle third (6 mm from the apex), as observed by the different filling techniques. Higher PGFA and PVFA values were obtained for G3 and G1, respectively. Both cold techniques (G1 and G3) promoted lower PSFA than both warm techniques (G2 and G4).
Conflict of Interest
None declared.

References

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