INTRODUCTION
The success of endodontic treatment depends on a sequence of clinical procedures, including cleaning, disinfecting, shaping and obturating. The obturation of the cleaned root canal should be performed to provide the best sealing possible in order to prevent bacterial leakage (1). Traditionally, the obturation is composed of a solid core material and a sealer that promotes the adhesion of the core to the dentin surfaces and also fills remote areas of the root canals due to its flowability (1). Complete obturation in complex anatomic configurations can be challenging due to the high incidence of irregularities such as isthmuses, fins and deltas, and these anatomical configurations can have a negative effect on the quality of the filling process (2-5). Furthermore, unfilled isthmus areas can house multi-species biofilms, which might lead to the failure of endodontic treatment and consequently to a possible retreatment (6).

Gutta-percha is the most used core in root canal filling because of its adequate dimensional stability. In addition, this material can be plasticised with the application of heat (7). The thermoplastici-
sation of gutta-percha can significantly increase its adaptation and filling capacity into the root canals (5). Several thermoplastic techniques have been proposed, and they are widely used to improve the root canal fillings (7-10). Buchanan (7) modified the vertical compaction technique using the System B (SybronEndo, Orange, USA) device to promote a continuous wave method for warm vertical condensation of gutta-percha. Thermafil (Dentsply Tulsa, Johnson City, USA) is another well-known method that consists of heated α-phase gutta-percha on a carrier for obturation (9). In line with those findings, an earlier study showed more gutta-percha, less sealer and fewer voids in mesial root canals of mandibular molars obturated with System B and Thermafil techniques in comparison to single cone and lateral condensation filling techniques (5).

Gutta-percha and epoxy resin-based root canal sealers are the most common obturation materials, but materials such as Resilon (Resilon Research LLC, Madison, USA) an polyester polymer-based in conjunction with dual-cured methacrylate sealer and self-etching primer have been proposed as substitutes for the traditional root canal filling (11). Resilon is available as cones according to International Organization for Standardization (ISO), similar to gutta-percha. Moreover, Resilon has a significantly higher thermal plasticity in comparison to gutta-percha (12). As was previously shown in a tooth model, this advantageous property improves its flow into root canal grooves and depressions (13). The gutta-percha and epoxy resin-based sealers can also promote better marginal adaptation of the root canal obturation compared to Resilon and methacrylate sealer, and this might be responsible for the formation of large gaps (14).

Although many studies have investigated the sealing ability of Resilon in single-rooted canals, none have evaluated the unfilled areas when using this material in complex morphologies of mesial root canals of mandibular molars (10). Additionally, Resilon’s thermoplasticity that allows it to flow into isthmuses during the warm vertical compaction or core carrier-based techniques compared to gutta-percha has not been reported so far. Thus, this in vitro study aimed to evaluate the obturation of mesial root canals of mandibular first molars performed with single cone, System B, and core carrier-based techniques using gutta-percha or Resilon.

Two null hypotheses were tested:

1. Thermoplastic techniques do not completely fill complex root canals systems.
2. There is no difference between the root canal filling materials (gutta-percha/epoxy sealer and Resilon/methacrylate sealer) regardless of the obturation technique.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of Bauru School of Dentistry, University of São Paulo (Protocol: CEP 122-2009), and the teeth were donated by the Bank of Teeth of the same institution, with consent of the patients allowing the use of their extracted teeth for research purposes.

Seventy-eight mesial roots of extracted human mandibular first molars with complete rhizogenesis, 19 to 21 mm length, curvature degrees between 15° and 30°, with two canals and separate foramina, and without previous endodontic treatment or calcifications were selected. High-speed diamond burs were used to form tooth-access cavities, and the working length was established by subtracting 1 mm of the total length up to the apex as measured with a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) (15). The root canals were prepared using K3 NiTi rotary system (SybronEndo) according to the manufacturer recommendations and the apical preparation was set up to size 35.04 rotary file. After the use of instruments, the root canals were irrigated with 1 mL of 2.5% sodium hypochlorite (NaOCl). A final irrigation was performed using a passive ultrasonic irrigation with a 20/0.01 ultrasonic file (Irrisonic E1; Helse, Santa Rosa de Viterbo, Brazil) with intermittent flushing for 1 min. The smear layer was removed with 2 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes, then the root canals were flushed with distilled water and dried with paper points.

Root canal obturation

The teeth were randomly distributed into 6 experimental groups (n=13 for each). ThermaSeal Plus (Dentsply) and Real Seal SE (SybronEndo) were the sealers used for the gutta-percha and Resilon fillings, respectively. Both sealers were mixed with Rhodamine B fluorescent dye (Sigma-Aldrich, St. Louis, USA) at an approximate concentration of 0.1% to allow confocal microscope visualisation (8, 16). When the Real Seal SE sealer was used, the specimens were light-cured for 40 seconds.

The single-cone technique was used for the Groups 1 and 2. For Group 1, 35.04 K3 gutta-percha cones (SybronEndo) with a tug-back were selected. The canals were first filled with ThermoSeal Plus sealer using a lentulo instrument, and then the selected gutta-percha cones were inserted. A compatible System B plugger was placed at the canal orifice and activated so that the gutta-percha was vertically condensed with a plugger at 1 mm below the canal orifice. In Group 2, a 35.04 Resilon cone with Real Seal SE sealer was used in the same manner as described for Group 1.

The vertical compaction technique using the System B device was used in Groups 3 and 4. In the Group 3, the first step of obturation was performed similar to Group 1, and the System B Elements were pre-set at 200°C and the down-pack procedure was performed using a 0.06 plugger inserted within 4 mm of the working length. At the apical level, the gutta-percha was condensed using Buchanan hand pluggers (SybronEndo). The extruder hand piece of the Elements was used as the backfill method. In Group 4, the Resilon and Real Seal SE were used in the same manner as described for Group 3; however, the System B was pre-set at 150°C and the Resilon was used for the backfill. A new layer of sealer was applied before the backfilling procedures in both groups.

For Group 5, the root canal fillings were size 35 Thermafil carrier points as determined with a Thermafil 35 verifier. The canals were filled with ThermaSeal Plus sealer with the use of a lentulo spiral filler. Then the Thermafil obturator was heated in the ThermaPrep Plus oven and inserted into the canal until
reaching the established working length. In the Group 6, the 0.35 mm RealSeal 1 was used in the same manner as described for Group 5, but the RealSeal SE sealer and the RealSeal 1 oven were used.

All specimens were stored at 37°C with 100% humidity for one week, and a single operator performed the obturation procedures.

**Sectioning, isthmus classification and microscopy analysis**

Each specimen was horizontally sectioned at 2 mm, 4 mm, and 6 mm from the apex using a 0.3 mm Isomet saw (Buehler, Lake Bluff, USA) under water cooling at 200 rpm. The slices were fixed on a glass plate and then polished with metallographic abrasive grinding with a sequence of SiC abrasive papers (320 grit, 600 grit, 800 grit, and 1200 grit). The specimens were evaluated with a stereomicroscope (Carl Zeiss, Jena, Germany) using 8x magnification and with a confocal laser scanning microscope (CLSM-Leica, Mannheim, Germany).

A high-resolution stereomicroscope camera was used to acquire the specimens’ sectional images. From the images, the presence of isthmuses was registered in five categories and their proportions among the experimental groups were systematically verified (17). The Axiosview software (Carl Zeiss, Jena, Germany) was used to measure the total area of the two mesial root canals, the gutta-percha or Resilon, and the voids. All obtained values were converted into mm², and the percentages (%) of gutta-percha/Resilon, sealer, and voids were calculated. The measurements were repeated twice to ensure consistency.

Confocal laser scanning microscopy images were obtained at 10 μm below the sample surface by using a 10× lens and a 1 μm step size (8). The images were acquired at 1024×1024 pixels and were evaluated using the Image J software (National Institutes of Health, Bethesda, USA). The total area of the root canals and the perimeter of dental tubules in which the sealer penetrated were measured to determine the sealer penetration, which was expressed in percentages.

**Statistical analysis**

The D’Agostino and Person normality tests did not show normal distributions in regard to the preliminary analysis of percentages of gutta-percha/Resilon, sealer, and voids data, so the statistical analysis was performed using the Kruskal-Wallis test with Dunn’s test for post hoc analysis. The percentage of sealer penetration was compared by ANOVA and Tukey tests. The significance was set at 5%, and the Prism 5.0 software (GraphPad, La Jolla, USA) was used as the analytical tool.

**RESULTS**

The Kruskal-Wallis test showed no significant differences for isthmus distribution or root canal diameter for any of the evaluated levels, thus there was a homogeneous distribution of the root canal anatomy among the experimental groups (Figure 1) (Table 1-3).

The median, minimal, and maximal values of the stereomicroscope data are shown in Table 1-3. Overall, Thermafil and Real Seal 1 showed a greater percentage of core material and less sealer (P<0.05) at the 2 mm level. The percentages of voids were similar (P>0.05) for all groups (Table 1). There was significantly more filling material in System B and carrier-based techniques in comparison to the single cone using gutta-percha or Resilon (P<0.05) at the 4 mm level (Table 2). For the 6 mm level, System B techniques showed better obturation performance.

### Table 1. Median, minimal, and maximal values of percentages of material, sealer, and voids in root canal obturations at the 2 mm level.

| Group       | Canal diameter | Gutta-percha/resilon (%) | Sealer (%) | Voids (%) |
|-------------|----------------|--------------------------|------------|-----------|
| Single-cone/GP | 0.55          | 56.98 (43.39-82.48)       | 39.62 (17.52-56.10) | 0.69 (0.9-15) |
| Single-cone/R | 0.49          | 59.28 (46.65-69.64)       | 37.96 (27.91-46.73) | 1.62 (12.97) |
| System B/GP  | 0.48          | 72.55 (54.01-86.55)       | 24.44 (10.31-45.99) | 1.53 (10.19) |
| System B/R   | 0.66          | 69.11 (43.20-80.52)       | 30.24 (19.48-55.50) | 1.30 (5-85)  |
| Thermafil    | 0.47          | 85.29 (68.74-91.59)       | 11.94 (8.27-30.09)  | 1.16 (7-0.2)  |
| Real seal    | 1.054         | 82.14 (74.88-94.68)       | 17.86 (5.32-25.12)  | 0.00 (0-4.02) |

**Table 2. Median, minimal, and maximal values of percentages of material, sealer, and voids in root canal obturations at the 4 mm level.**

| Group       | Canal diameter | Gutta-percha/resilon (%) | Sealer (%) | Voids (%) |
|-------------|----------------|--------------------------|------------|-----------|
| Single-cone/GP | 1.07          | 46.67 (31.97-68.98)       | 45.12 (26.46-58.31) | 3.54 (0-23.81)  |
| Single-cone/R | 1.02          | 49.52 (27.18-81.40)       | 45.92 (16.28-68.87) | 2.33 (0-13.87)  |
| System B/GP  | 0.83          | 86.57 (70.98-91.86)       | 13.15 (8.14-25.69)  | 0.00 (11.35)   |
| System B/R   | 1.03          | 87.94 (84.08-93.59)       | 10.27 (6.41-13.47)  | 0.00 (0-1.90)  |
| Thermafil    | 0.89          | 79.53 (68.88-90.91)       | 19.48 (7.58-33.70)  | 0.78 (5-25)    |
| Real seal    | 1.08          | 82.99 (75.52-93.08)       | 16.11 (6.92-23.89)  | 0.13 (0-3.76)  |

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Single-cone/GP: Single-cone technique with gutta-percha, Single-cone/R: Single-cone technique with Resilon, System B/GP: Vertical compaction technique of warm gutta-percha using the System B device, System B/R: Vertical compaction technique of warm Resilon using the System B device.
The mesial roots of mandibular molars do not follow a consistent pattern and have a high incidence of isthmuses, and this can influence the quality of the root canal filling (2, 4, 5, 17). Thus, the distribution of isthmuses in the root canals was verified, and similarity between groups was observed. In addition, the root canal areas were also similar showing a homogeneouse experimental model. The method of root sectioning for microscopic observations provides relevant data such as the homogeneity, adaptation to dentin, filling ability, the presence of voids, and the intratubular penetration of sealer, therefore the combined use of stereomicroscope and confocal laser scanning microscope methods allows for the collection of accurate data (7, 8, 12, 18, 19).

Regardless of the material used, no technique provided complete root canal filling. Thus, the first null hypothesis was accepted. The single cone fillings were tested in this study with the absence of compaction forces to establish a control group. At the 2 mm level, this technique was able to promote a similar obturation technique. Figure 2 shows representative stereomicroscope and confocal images of the obturated areas of the root canals at different levels.

**DISCUSSION**

The mesial roots of mandibular molars do not follow a consistent pattern and have a high incidence of isthmuses, and this can influence the quality of the root canal filling (2, 4, 5, 17). Thus, the distribution of isthmuses in the root canals was verified, and similarity between groups was observed. In addition, the root canal areas were also similar showing a homogeneous experimental model. The method of root sectioning for microscopic observations provides relevant data such as the homogeneity, adaptation to dentin, filling ability, the presence of voids, and the intratubular penetration of sealer, therefore the combined use of stereomicroscope and confocal laser scanning microscope methods allows for the collection of accurate data (7, 8, 12, 18, 19).

Regardless of the material used, no technique provided complete root canal filling. Thus, the first null hypothesis was accepted. The single cone fillings were tested in this study with the absence of compaction forces to establish a control group. At the 2 mm level, this technique was able to promote a similar filling as the System B technique as previously described (5,

**TABLE 3.** Median, minimal, and maximal values of percentages of material, sealer, and voids in root canal obturations at the 6 mm level. Different letters in each column indicate statistically significant differences (P<0.05)

| Group               | Canal diameter | Gutta-percha/resilon (%) | Sealer (%) | Voids (%) |
|---------------------|----------------|--------------------------|------------|-----------|
| Single-cone/GP      | 1.24<sup>a</sup> | 50.78 (39.22-74.03)<sup>a</sup> | 44.67 (32.34-59.02)<sup>a</sup> | 3.06 (0-16.19)<sup>a</sup> |
| Single-cone/R       | 1.17<sup>a</sup> | 64.41 (41.46-79.78)<sup>ab</sup> | 34.85 (19.12-53.78)<sup>ab</sup> | 1.19 (0-5.88)<sup>ab</sup> |
| System B/GP         | 1.18<sup>a</sup> | 88.45 (77.07-93.06)<sup>abcd</sup> | 11.51 (6.94-22.32)<sup>abcd</sup> | 0.00 (0-1.35)<sup>b</sup> |
| System B/R          | 1.31<sup>a</sup> | 92.17 (83.74-95.45)<sup>d</sup> | 7.83 (4.55-16.26)<sup>d</sup> | 0.00 (0-1.16)<sup>c</sup> |
| Thermafil           | 1.17<sup>a</sup> | 77.87 (69.61-90.12)<sup>bcd</sup> | 20.92 (9.16-30.39)<sup>bcd</sup> | 0.47 (0-7.65)<sup>b</sup> |
| Real seal           | 1.16<sup>a</sup> | 86.47 (63.90-93.38)<sup>abcd</sup> | 13.54 (6.11-29.82)<sup>abcd</sup> | 0.87 (0-7.49)<sup>b</sup> |

Single-cone/GP: Single-cone technique with gutta-percha, Single-cone/R: Single-cone technique with Resilon, System B/GP: Vertical compaction technique of warm gutta-percha using the System B device, System B/R: Vertical compaction technique of warm Resilon using the System B device

**TABLE 4.** Percentage of sealer penetration (mean and standard deviation) at different root canal levels. The same letters in the same column indicate no statistical difference (P<0.05)

| Group               | 2 mm level                  | 4 mm level                  | 6 mm level                  |
|---------------------|-----------------------------|-----------------------------|-----------------------------|
| Single-cone/GP      | 44.04 (34.53)<sup>a</sup>   | 36.48 (17.24)<sup>a</sup>   | 53.05 (21.61)<sup>a</sup>   |
| Single-cone/R       | 35.63 (24.26)<sup>a</sup>   | 38.24 (18.65)<sup>a</sup>   | 52.47 (24.01)<sup>a</sup>   |
| System B/GP         | 58.69 (17.75)<sup>a</sup>   | 53.82 (24.24)<sup>a</sup>   | 69.23 (13.47)<sup>a</sup>   |
| System B/R          | 41.34 (28.99)<sup>a</sup>   | 56.73 (26.82)<sup>a</sup>   | 67.73 (21.16)<sup>a</sup>   |
| Thermafil           | 40.98 (18.98)<sup>a</sup>   | 47.17 (20.75)<sup>a</sup>   | 62.61 (19.74)<sup>a</sup>   |
| Real seal           | 1.62 (7.66)<sup>a</sup>     | 63.28 (14.82)<sup>a</sup>   | 70.14 (19.95)<sup>a</sup>   |

Single-cone/GP: Single-cone technique with gutta-percha, Single-cone/R: Single-cone technique with Resilon, System B/GP: Vertical compaction technique of warm gutta-percha using the System B device, System B/R: Vertical compaction technique of warm Resilon using the System B device

**Figure 1.** Distribution of isthmuses according to Teixeira (12) at the 2 mm (a), 4 mm (b), and 6 mm (c) level from the apex. Graphic representation showing the root canal configurations. Type I: defined as either two or three canals with no notable communication. Type II: defined as two canals with a definite connection between the two main canals. Type III: defined as three canals with a definite connection between the three main canals. Type IV: defined as canals extending into the isthmus area. Type V: defined as a true connection or corridor throughout the section. G1 (single-cone gutta-percha), G2 (single-cone Resilon), G3 (System B/gutta-percha), G4 (System B/Resilon), G5 (Thermafil) and G6 (Real Seal 1)
In a previous retrospective in vivo study, the root canal filling performed with Thermafil and using warm vertical compaction showed 80% and 42% extruded filling material, respectively (22).

In this context, the physicochemical properties of the sealers are relevant primarily for single-cone techniques. The flow-ability of both sealers appears to be comparable because they were able to fill areas with isthmuses that are normally difficult to address with the rotary shaping techniques used. In the present study the void areas were similar for all groups, and this was similar to what was previously found in curved root canals (23). Solubility is another critical factor when single-cone techniques are considered for clinical use because the critical area of fillings is located at the sealer-dentin interface (24).

Both core carrier techniques (Thermafil and Real Seal 1) showed lower amounts of sealer, as reported previously (9, 20). Although the core carrier techniques provide better sealing at 2 mm, some studies have reported the risk of root canal filling extrusion. In a previous retrospective in vivo study, the root canal filling performed with Thermafil and using warm vertical compaction showed 80% and 42% extruded filling material, respectively (22).
ing to Resende et al. (25), both the methacrylate sealer and epoxy resin sealer used in the present study have a similar solubility. In contrast, De-Deus et al. (18) showed that the Resilon and self-etching sealer induce a high incidence of gaps. In the present study, we found a similar distribution of sealer inside the root canal anatomy using both sealers. The sealer interface under stressed conditions can be expected to be different. In a previous study, the gutta-percha and epoxy resin-based sealer showed better marginal adaptation in comparison to Resilon and methacrylate sealer filled with single cone and System B techniques (14).

The intensity of heat transferred through a material is significant when considering thermoplastic obturation. According to a previous study, gutta-percha and Resilon cones have a similar thermoplastic ability (26). However, Tanomaru-Filho et al. (12) showed that Resilon has a significantly higher thermoplasticity in comparison to gutta-percha. Despite the difference in the temperature used to reach the thermoplastic abilities in both materials, we found no significant difference in obturation for the two types of materials or sealers tested, which was in concordance with previous studies (13, 27). The System B technique provided fewer voids at the 4 mm and 6 mm levels compared to the single-cone technique, thus increasing the quality of obturations, but in contrast to our results a previous study with a similar methodology found no significant differences between these techniques in terms of the percentage of voids (23).

According to the intratubular sealer penetration parameter found in the CLSM data, both sealers showed similar performance for the three evaluated techniques. These results represent an important property because sealer penetration inside the dentinal tubules might act as a physical barrier to separate any residual microorganisms from nutrient sources (8).

The second null hypothesis was also confirmed because the results obtained in this study showed that root canal filling techniques using gutta-percha or Resilon had a similar performance; in other words, both of them presented similar degrees of filling areas using System B or the carrier-based techniques. The superiority of gutta-percha filling in comparison to Resilon shown in previous studies can be explained by other factors not related to the filling techniques, such as the physicochemical properties of the sealer such as polymerisation shrinkage, extended setting time, or solubility (23, 28-30). These factors show that Resilon fillings reach their weakest point at the sealer interface. Regardless of the materials used, better filling quality was obtained with the thermoplasticised techniques that are able to fill the isthmus area. Therefore, such techniques are indicated to fill the complex root canal anatomy.

**CONCLUSION**

None of the materials or techniques tested here completely filled the mesial root canals of mandibular molars; however, the plasticised techniques were more efficient. The obturations using both gutta-percha/ThermaSeal Plus and Resilon/Real Seal SE were comparable regardless of the technique used.
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