Fatigue behavior of teeth filled with calcium silicate- and epoxy resin-based sealers in bulk or associated with a main core material

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Abstract

Objective:
To evaluate the fatigue behavior of teeth filled with a calcium silicate-based sealer (Bio-C Sealer, BC) in comparison to an epoxy resin-based sealer (AH Plus, AH), in bulk or associated with gutta-percha as main core material.

Methods:
Root canals of 72 human maxillary incisors and canines were prepared using nickel-titanium reciprocating instruments and randomly assigned to 2 control groups: C- (without preparation and filling) and C+ (prepared but not filled); and 4 experimental groups: BC-B (BC in bulk); BC-GP (BC + gutta-percha); AP-B (AH in bulk); AP-GP (AH + gutta-percha). The specimens were submitted to a survival analysis after the cyclic fatigue test.

Results:
The experimental groups showed similar fatigue behavior (P>.05), being also similar to C+ (BC-B = BC-GP = AP-B = AP-GP = C+). The C- group presented the best fatigue performance (P<.05), being similar only to the AP-GP group (P>.05). Considering a premature failure rate (~10%), lower performance is observed for experimental groups filled in bulk. Conclusions:
The use of calcium silicate-based sealer is valid, both as bulk or associated with gutta-percha as a main core material, as it resulted in similar mechanical performance to an epoxy resin-based sealer. Despite that, the use of gutta-percha, as a main core material, seems to reduce the risk of premature failures. Clinical relevance: Ah Plus associated with gutta percha as main core material show similar fatigue behavior to sound teeth. Teeth filled in bulk, without gutta-percha as main core, had higher risk of premature failures.

1 INTRODUCTION

Root fracture (RF) is one of the possible consequences of endodontic treatment and it is an unfortunate event of challenging diagnosis, which usually condemns the tooth to extraction [1–3]. Potential causes of RF include excessive loss of tooth structure due to caries, trauma and endodontic procedures, such as root canal access and instrumentation or exaggerated stress caused during lateral condensation of gutta-percha [4]. Therefore, sealing the root canal with biocompatible materials that improve the strength of the remaining structure would be a primary purpose of the endodontic filling [5].

The standard obturation technique uses an endodontic sealer associated with a main core material [6]. Gutta-percha is a thermoplastic material widely used to fill root canals due to its good biological behavior and low allergenic potential; it is also easily removed in cases of endodontic re-intervention [7]. However, its low elastic modulus, associated with its inability to establish a chemical bond to the dentin walls, makes the use of endodontic sealers in association with gutta-percha essential [8, 9]. The sealers promote the union between the main core material (gutta-percha) and the root canal walls [10]. Furthermore, they have the ability to penetrate into dentinal tubules, establishing a micromechanical interlocking that might increase the fatigue resistance of the roots [5, 11]. AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) is an epoxy resin sealer considered the "gold standard" for root canal filling [12] due to its excellent physicochemical [13] and satisfactory biological [14] properties. Studies have already demonstrated that AH Plus increases the fracture resistance of root-canal treated teeth when compared to other materials [5, 15].

Currently, tricalcium silicate-based sealers are available in the market, recognized due to their biocompatibility, antimicrobial and bioactive properties [16, 17]. Some manufacturers indicate that calcium silicate sealers could be successfully used alone in the root canal space as a single root filling material (i.e., in bulk) or associated with a main core material like gutta-percha [18, 19]. According to Nagas et al. (2014), in the first scenario, the adhesion to dentin would occur more homogeneously through the formation of a "monoblock" along the root canal, increasing the dislocation resistance of the filling [19]. Bio-C Sealer (Angelus; Londrina, Paraná, Brazil) is an injectable and pre-mixed calcium silicate sealer that requires moisture from the dentinal tubules to promote the total setting [20]. A few studies have evaluated its physicochemical [21, 22] and biological [20, 23] characteristics, but there is still a lack of scientific research regarding the fatigue behavior of teeth filled with this new material.

In addition, few studies have explored root canal obturation using endodontic sealers in bulk, without a main core material [6, 19, 24]. Thus, the aim of the present in vitro study was to evaluate the fatigue behavior of root-canal treated teeth filled by the single cone technique (with gutta-percha) or by the "in bulk" technique (without gutta-percha) with the new endodontic sealer (Bio-C Sealer), compared to the "gold standard" (AH Plus). The null hypothesis is that there will be no difference between the fatigue resistance values with the different sealers and filling techniques.

2 MATERIALS AND METHODS

2.1. Study design

The manuscript was written based on the 'Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021' guideline [25]. The study was submitted and approved by the Institutional Ethics Committee (CAAE 30345120.7.0000.5346).

The sample size calculation was performed based on the parameters described by Patil et al. [3] (monotonic test): fatigue failure load of 332 (± 17) N for group 1 (AH Plus) and 354 (± 20) N for group 2 (Bio-C Sealer); 80% power; 5% significance level (OpenEpi 3.01, Atlanta, GA). Therefore, the estimated minimum sample size was found to be 12 teeth per group (n = 12).
This study was conducted with two control groups established based on previous study designs [3, 15], and four experimental groups, considering the “endodontic sealer” factor in two levels (AH-Plus and Bio-C Sealer) and the “filling technique” factor in two levels (with or without gutta-percha). Thus, teeth were randomly divided into six groups as follows (N = 72): C: no intervention; C+: root canal preparation but no filling; BC-B: Root canal filling using Bio-C Sealer in bulk; BC-GP: Root canal filling using Bio-C Sealer and the single cone technique (Reciproc R50 gutta-percha point; VDW); AP-B: Root canal filling using AH Plus sealer in bulk; AP-GP: Root canal filling using AH Plus sealer and the single cone technique (Reciproc R50 gutta-percha point; VDW).

### 2.2. Specimen preparation

Seventy-two human maxillary incisors and canines with complete root formation and straight roots (< 5°) were used. To confirm the presence of a single root canal, the teeth were submitted to digital periapical radiographs (RVG 5100; Carestream Health, Rochester, NY) in the buccolingual (BL) and mesiodistal (MD) directions. With the aid of a digital measurement tool (ImageJ; U.S. National Institutes of Health, Bethesda, MD), the BL and MD dimensions were obtained and the average values were calculated. Teeth that showed measurements 15% greater than the average were discarded to obtain a more homogeneous sample [5]. The external root surfaces were cleaned with periodontal scalers (Golgran, São Paulo, SP, Brazil) and analyzed under a digital stereomicroscope (StereoDiscovery V20, Zeiss, Oberkochen, Germany) with 20× magnification to verify the presence of pre-existing defects. In case of cracks or fracture lines, the teeth were excluded and replaced. Then, a stratified randomization was performed considering the factor “type of tooth” (incisor or canine) and the selected teeth were randomly allocated into the different groups.

### 2.3. Root canal instrumentation

All root canals were prepared by a single operator (L.C.C). After endodontic access, the working length (WL) was determined by subtracting 1 mm from the length of a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) with its tip visualized at the apical foramen. Root canal preparation was performed using nickel-titanium reciprocating instruments (Reciproc R50; VDW, Munich, Germany) in the mode “Reciproc All” on an endodontic motor (VDW Silver; VDW, Munich, Germany). In-and-out movements with an amplitude of 3 mm were applied in the cervical, middle and apical root thirds until reaching the WL. Throughout instrumentation, the root canals were irrigated with 20 ml of 2.5% sodium hypochlorite solution (NaOCl; Asfer Indústria Química, São Caetano do Sul, SP, Brazil), followed by 2 ml of 17% EDTA (Biodinâmica, Ibiporá, PR, Brazil) for smear layer removal. Then, 5 ml of saline solution (Farmax, Divinópolis, MG, Brazil) were used as final irrigation.

After root canal preparation, the specimens were embedded in polyvinyl chloride (PVC) cylinders (Dencrilay; Dencril, Caieiras, SP, Brazil) (20 mm x 25 mm) filled with a chemically cured acrylic resin (Clássico, Campo Bom Paulista, SP, Brazil), as previously described by Osiri et al. [5]. The specimens were fixed in a parallellometer, with the long root axis of the teeth and cylinder parallel to each other and perpendicular to the ground. Then, the acrylic resin was poured inside the cylinder up to 3 mm from the cement-enamel junction.

### 2.4. Root canal filling

For the groups filled with AH Plus, the root canals were totally dried with #50 absorbent paper points (Tanari, Manacapuru, AM, Brazil). On the other hand, for the groups filled with Bio-C Sealer, the canals were dried with only one absorbent paper point to maintain residual moisture [26]. Endodontic sealers and their compositions are described in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH Plus Jet</td>
<td>Paste A: Bisphenol A epoxy resin; bisphenol-F epoxy resin; calcium tungstate; zirconium oxide; iron oxide and silica. Paste B: adamantized amine; N, N'-dibenzyl-5-oxanonanedianmine 1,9; TCD-diamine; calcium tungstate; zirconium oxide; silicone oil and silica.</td>
<td>Dentsply; DeTrey GmbH, Konstanz, Germany.</td>
</tr>
<tr>
<td>Bio-C Sealer</td>
<td>Tricalcium silicate; dicalcium silicate; tricalcium aluminate; calcium oxide; zirconium oxide; silicon oxide; polyethylene glycol; iron oxide.</td>
<td>Angelus; Londrina, Paraná, Brazil.</td>
</tr>
</tbody>
</table>

In the BC-B and BC-GP groups, Bio-C Sealer was introduced into the root via an intracanal tip provided by the manufacturer. Then, a #20 K-file was used to spread the sealer on the canal walls with pumping movements, followed by a Lentulo spiral (N° 3; Dentsply-Maillefer, Ballaigues, Switzerland) at low speed, until the root was completely filled [27]. Additionally, only in the BC-GP group, a Reciproc R50 gutta percha point (VDW), with a good tug-back, was embedded with the sealer and slowly inserted into the canal until it reached the WL.

In the AP-B and AP-GP groups, AH Plus was dispensed directly into the root canal from the double-barrel mixing syringe via an intracanal tip attached to the auto-mixing tip provided by the manufacturer. Next, the sealer was spread on the canal walls with the aid of an #20 K-file and a Lentulo spiral, as previously described. Additionally, only in the AP-GP group, a Reciproc R50 gutta percha point (VDW) with a good tug-back and coated with sealer was slowly introduced up to the WL.

Mesiodistal and buccolingual digital periapical radiographs were taken to ensure no empty spaces in the obturation mass. After the complete setting of the sealer, the specimens were restored. First, the endodontic access was etched with 37% phosphoric acid for 15 seconds, then rinsed and gently air-dried, followed by the application of a bond agent (Single Bond; 3M ESPE, St Paul, MN). Then, a composite resin (Filtek Z350 XT; 3M, Sumaré, São Paulo, Brazil) was inserted by the incremental technique and photocured for 30 seconds (palatal side) [28].

### 2.5. Cyclic fatigue testing
All specimens were submitted to the cyclic fatigue testing in an electro-dynamic testing machine (Instron ElectroPlus E3000; Instron Corporation, Norwood, MA). A 45º angle load was applied by a 6-mm-diameter stainless-steel sphere, directly positioned into the lingual/palatal surface while the specimens were submerged in distilled water, as previously described by Missau et al. [28]. The fatigue test was performed with an initial load of 100 N at a frequency of 20 Hz for 5,000 cycles (to ensure the correct positioning of the piston over the specimen), followed by increments of 25 N for 10,000 cycles in each step, until the specimen failure (complete fracture). The fatigue failure load (FFL) and the number of cycles for failure (CFF) were recorded for statistical analysis.

2.6. Failure mode

For fracture morphology analysis, the specimens were analyzed under a stereomicroscope (Stereo Discovery V20, Zeiss, Gottingen, Germany) under 4× magnification by a trained and calibrated examiner (L.C.C). The training consisted of an expository lecture by an experienced examiner (I.M.L). Calibration was performed using 20% of the study sample. Inter- and intra-examiner agreement was calculated using Cohen’s kappa coefficient (> 0.76).

The failure mode was classified according to the following criteria adapted from Missau et al. (26): mode I, fracture above the cement-enamel junction (repairable); or mode II, fracture below the cement-enamel junction (irreparable). Representative images of the failure modes were obtained using a digital camera (Fig. 1).

2.7. Statistical analysis

Data of FFL (in Newtons) and CFF (in counts) were analyzed by Kaplan-Meier and Mantel-Cox (Log Rank) tests (P < .05), and the survival rates were tabulated for each step of the fatigue test. Data analysis was performed using the SPSS Statistics v. 21 software (IBM Analytics, Chicago, IL). Regarding failure mode, data were described qualitatively and a chi-square test was applied to evaluate the association between endodontic sealer or filling technique and the type of fracture.

3 RESULTS

Table 2 presents the results of FFL and CFF in each group. There was a significant difference (P < .05) between the studied conditions, where the C- group showed higher FFL and CFF results. When the root canal treatment was performed using the "gold standard" protocol (AP-GP group), it was noticed a similar fatigue behavior compared to healthy teeth (C- group) (P > .05). However, no difference was found among AP-GP and the other experimental groups and non-filled teeth (C+ group).

<table>
<thead>
<tr>
<th>Groups</th>
<th>FFL (N)</th>
<th>95% CI</th>
<th>CFF</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC-B</td>
<td>427.0 ± 139.5</td>
<td>338.4 – 515.7</td>
<td>100,450 ± 57,389</td>
<td>63,986 – 136,914</td>
</tr>
<tr>
<td>BC-GP</td>
<td>414.5 ± 139.9</td>
<td>325.6 – 503.5</td>
<td>93,795 ± 56,471</td>
<td>57,915 – 129,675</td>
</tr>
<tr>
<td>AP-B</td>
<td>370.8 ± 87.1</td>
<td>315.4 – 425.2</td>
<td>77,972 ± 35,910</td>
<td>55,156 – 100,789</td>
</tr>
<tr>
<td>AP-GP</td>
<td>525.0 ± 231.1</td>
<td>378.1 – 671.8</td>
<td>140,666 ± 94,350</td>
<td>80,718 – 200,613</td>
</tr>
<tr>
<td>C+</td>
<td>450.0 ± 81.1</td>
<td>398.4 – 501.5</td>
<td>109,953 ± 31,446</td>
<td>89,972 – 129,933</td>
</tr>
<tr>
<td>C-</td>
<td>687.5 ± 206.5</td>
<td>556.2 – 818.7</td>
<td>203,177 ± 83,114</td>
<td>150,369 – 255,986</td>
</tr>
</tbody>
</table>

Different uppercases in the columns mean statistically significant difference (Kaplan-Meier and Mantel-Cox post-hoc tests) for FFL and CFF (p< 0.05).

It was observed that BC-B, AP-B and BC-GP groups presented a higher risk of premature failure compared to C+, C- and AP-GP groups since they presented, respectively, 25%, 17% and 17% probability of failure at step 275 N (45,000 cycles), while the latter still held 0% probability of failure (Table 3). Furthermore, it was found that, with the advancement of the fatigue test, at step 475 N (125,000 cycles), all groups that received endodontic preparation showed similar results for probability of failure (ranging from 17 to 33% probability of survival). In contrast, the group that did not receive the intervention (C-) had a survival rate of 100%. In addition, at step 625 N (185,000 cycles), only AP-GP and C- groups presented a considerable survival (33 – 42%), while the AP-B group already had a 100% probability of failure at step 500 N (135,000 cycles).
Table 3 Survival rate (probability of the specimen to survive without fracture to the respective Fatigue Failure Load – FFL in Newtons and Number of Cycles F – CFF) for the experimental groups and respective standard errors.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FFL (N) / CFF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100/ 200/ 225/ 250/ 275/ 300/ 325/ 350/ 375/ 400/ 425/ 450/ 475/ 500/</td>
</tr>
<tr>
<td></td>
<td>5,000 15,000 25,000 35,000 45,000 55,000 65,000 75,000 85,000 95,000 105,000 115,000 125,000 135,000</td>
</tr>
<tr>
<td>BC-B</td>
<td>1 1 0.92 0.83 0.75 0.75 0.67 0.67 0.58 0.58 0.50 0.42 0.33 0.33</td>
</tr>
<tr>
<td></td>
<td>(0.08) (0.11) (0.12) (0.12) (0.13) (0.13) (0.14) (0.14) (0.14) (0.14) (0.14) (0.13) (0.13)</td>
</tr>
<tr>
<td>BC-GP</td>
<td>1 1 1 0.92 0.83 0.83 0.75 0.66 0.66 0.42 0.17 0.17 0.17 0.08</td>
</tr>
<tr>
<td></td>
<td>(0.08) (0.11) (0.11) (0.12) (0.13) (0.13) (0.14) (0.14) (0.11) (0.11) (0.11) (0.08)</td>
</tr>
<tr>
<td>AP-B</td>
<td>1 1 1 0.92 0.83 0.75 0.50 0.42 0.42 0.33 0.25 0.25 0.17 0.00</td>
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<tr>
<td></td>
<td>(0.08) (0.12) (0.14) (0.14) (0.13) (0.12) (0.12)</td>
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<tr>
<td>AP-GP</td>
<td>1 1 1 1 1 0.92 0.75 0.67 0.67 0.58 0.58 0.42 0.33 0.33</td>
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<tr>
<td></td>
<td>(0.08) (0.12) (0.13) (0.13) (0.14) (0.14) (0.14) (0.13)</td>
</tr>
<tr>
<td>C+</td>
<td>1 1 1 1 1 1 0.92 0.83 0.83 0.75 0.58 0.25 0.25 0.17</td>
</tr>
<tr>
<td></td>
<td>(0.08) (0.11) (0.12) (0.14) (0.12) (0.12) (0.11)</td>
</tr>
<tr>
<td>C-</td>
<td>1 1 1 1 1 1 1 1 1 1 1 1 1 1 0.92</td>
</tr>
<tr>
<td></td>
<td>(0.08)</td>
</tr>
</tbody>
</table>

* : / indicates no survivor specimen in the respective step

Continuation of Table 3.

<table>
<thead>
<tr>
<th>Groups</th>
<th>FFL (N) / CFF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>550/ 575/ 600/ 625/ 650/ 675/ 700/ 725/ 750/ 775/ 800/ ... 1050/ 1</td>
</tr>
<tr>
<td></td>
<td>155,000 165,000 175,000 185,000 195,000 205,000 215,000 225,000 235,000 245,000 255,000 355,000</td>
</tr>
<tr>
<td>BC-B</td>
<td>0.25 0.08 0.08 0.08 0.08 0.00 - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>(0.12) (0.08) (0.08) (0.08)</td>
</tr>
<tr>
<td>BC-GP</td>
<td>0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.00 - - - -</td>
</tr>
<tr>
<td></td>
<td>(0.08) (0.08) (0.08) (0.08) (0.08) (0.08) (0.08) (0.08) (0.08)</td>
</tr>
<tr>
<td>AP-B</td>
<td>- - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>AP-GP</td>
<td>0.33 0.33 0.33 0.33 0.33 0.33 0.25 0.17 0.08 0.08 0.08 ... 0.00 -</td>
</tr>
<tr>
<td></td>
<td>(0.13) (0.13) (0.13) (0.13) (0.13) (0.13) (0.12) (0.11) (0.08) (0.08) (0.08)</td>
</tr>
<tr>
<td>C+</td>
<td>0.08 0.08 0.08 0.00 - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>(0.08) (0.08)</td>
</tr>
<tr>
<td>C-</td>
<td>0.58 0.58 0.42 0.42 0.42 0.42 0.33 0.33 0.17 0.17 0.17 ... 0.00 -</td>
</tr>
<tr>
<td></td>
<td>(0.14) (0.14) (0.14) (0.14) (0.14) (0.13) (0.13) (0.13) (0.11) (0.11) (0.11)</td>
</tr>
</tbody>
</table>

* : / indicates no survivor specimen in the respective step
*** : / indicates absence of specimen failure in the respective step for each condition.

Table 4 indicates the fracture analysis, which showed that irreparable failure was the main mode in all experimental groups.

Table 4 – Failure mode [n(%)] in each experimental group.
of empty spaces in the root canal filling could induce the occurrence of early failures. Spreading of the sealer along the root canal walls, facilitating the appearance of bubbles in the obturation mass. When subjected to cyclic loads, the presence over time approximately the double of the load 300 N (55,000 cycles).

In the present study, teeth filled with AH Plus (an epoxy resin-based sealer) and gutta-percha (AP-GP group) showed the best fatigue behavior, similar to teeth that did not receive any intervention (group C). These results were also found in a previous study [15, 28], which demonstrated that AH-Plus in association with a gutta-percha point can enhance the fatigue resistance of root-canal treated teeth. Based on that, the filling procedure with AH Plus sealer and the single cone technique improved the mechanical performance of the teeth, producing a result similar to the observed in sound teeth.

Sealers containing calcium silicate represent an important alternative to filling procedures nowadays, especially due to their remarkable biocompatibility. Another feature that made calcium silicate-based sealers so popular is the potential ability to form a chemical bonding to dentin [32, 33]. Although the exact mechanism is still unclear, the nanoparticles present in these materials may allow it to flow into dentinal tubules forming interlocking bonds and establishing a mineral infiltration zone with the posterior formation of hydroxyapatite [33]. Recent studies have demonstrated that Bio-C Sealer showed better cytocompatibility, mineralization capacity [20], higher penetration and better adaptation to the dentinal tubules [34] compared to other filling materials. Moreover, it was shown that teeth filled with calcium silicate-based sealers presented higher values for fracture resistance when compared to those filled with AH Plus [3, 15]. These findings contradict those described in our study, where teeth filled with Bio-C Sealer presented a similar mechanical behavior to AP groups. When compared to non-prepared teeth, filling with Bio-C sealer showed significantly worse fatigue behavior. This may be explained by the different methodologies applied since most studies regarding mechanical behavior of root filled teeth use static load tests [3, 15, 35]. One of the highlights of the present study was the use of a cyclic fatigue test to assess the mechanical behavior.

Tooth fracture is a consequence of cyclic fatigue that happens in the oral cavity in response to the stress caused during mastication. In this situation, failure happens in a much lower load than the load capacity [36]. The cyclic fatigue test used here simulates, under controlled parameters such as number of cycles, frequency, and load, the intermittent loading movements observed in the mouth [28]. Thus, cyclic fatigue tests provide results replicating the clinical conditions and the cyclic nature of chewing. Studies using static loading only provide the maximum critical stress through the increased applied load, which does not allow predicting failures over time, since they do not simulate the stimuli of the oral cavity [28, 36].

Due to its potential ability to bond to root dentin, the manufacturers claim calcium silicate-based sealers perform successfully regardless of using a main core material [19]. This kind of obturation would create a single interface between the root filling material and root dentin, forming a “monoblock” along the root canal walls, avoiding the creation of gaps between gutta-percha points and sealer [19]. In that regard, previous studies have reported that root canal sealers showed higher push-out bond strength values when the obturation was performed in bulk than when it was associated with a master gutta-percha point [6, 19, 24]. Despite these favorable findings, herein, the teeth filled without gutta-percha (AP-B and BC-B groups) showed a similar mechanical behavior compared to those with gutta-percha (AP-GP and BC-GP groups). Also, it is fundamental to consider the early failures evidenced in the groups without gutta-percha (BC-B and AP-B), starting to fail in steps 225 N (25,000 cycles) and 250 N (35,000 cycles), respectively. In contrast, the AP-GP groups started to present failures at approximately the double of the load 300 N (55,000 cycles).

Those early failures present in groups filled without gutta-percha could be justified by a thicker layer of sealer, which can shrink during setting and dissolve over time [24]. These findings contradict the possible advantage of the obturation in bulk once the absence of a main core material may compromise the spreading of the sealer along the root canal walls, facilitating the appearance of bubbles in the obturation mass. When subjected to cyclic loads, the presence of empty spaces in the root canal filling could induce the occurrence of early failures.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reparable</th>
<th>Irreparable</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mode I</td>
<td>Mode II</td>
<td></td>
</tr>
<tr>
<td>BC-B</td>
<td>3 (25)</td>
<td>9 (75)</td>
<td>P≤ 0.05</td>
</tr>
<tr>
<td>BC-GP</td>
<td>1 (8.3)</td>
<td>11 (92)</td>
<td></td>
</tr>
<tr>
<td>AP-B</td>
<td>2 (17)</td>
<td>10 (83.3)</td>
<td></td>
</tr>
<tr>
<td>AP-GP</td>
<td>1 (8.3)</td>
<td>11 (91.7)</td>
<td></td>
</tr>
<tr>
<td>C+</td>
<td>0 (0)</td>
<td>12 (100)</td>
<td></td>
</tr>
<tr>
<td>C-</td>
<td>0 (0)</td>
<td>12 (100)</td>
<td></td>
</tr>
</tbody>
</table>

**4 DISCUSSION**

Root-canal treated teeth are more susceptible to root fracture than teeth without endodontic treatment [4, 29]. In this context, endodontic sealers are used to promote reinforcement of the remaining structure, adhering to the root canal surface, in an attempt to contribute to the long-term permanence of these teeth [3, 15]. Thus, the present study evaluated the fatigue behavior of teeth filled with two different endodontic sealers (Bio-C Sealer and AH Plus), with or without a main core material (gutta-percha). Indeed, it was observed that the endodontic access and preparation might induce damage to the root that can only be attenuated by the materials and filling techniques explored. In this sense, the null hypothesis was accepted since no significant difference was found among the experimental groups. However, the AP-GP group produced fatigue behavior similar to the group with no treatment (C-), which had the best fatigue performance.

In order to maintain a tridimensional seal within the root canal system, a filling material must adhere to the dentin walls [30]. Many studies have shown that epoxy resin-based sealers result in a high bond strength to the root canal dentin [19], penetrating into micro-irregularities and partially filling dentinal tubules [28], thus increasing mechanical retention and resistance to shear forces [30, 31]. In the present study, teeth filled with AH Plus (an epoxy resin-based sealer) and gutta-percha (AP-GP group) showed the best fatigue behavior, similar to teeth that did not receive any intervention (group C). These results were also found in a previous study [15, 28], which demonstrated that AH-Plus in association with a gutta-percha point can enhance the fatigue resistance of root-canal treated teeth. Based on that, the filling procedure with AH Plus sealer and the single cone technique improved the mechanical performance of the teeth, producing a result similar to the observed in sound teeth.

Sealers containing calcium silicate represent an important alternative to filling procedures nowadays, especially due to their remarkable biocompatibility. Another feature that made calcium silicate-based sealers so popular is the potential ability to form a chemical bonding to dentin [32, 33]. Although the exact mechanism is still unclear, the nanoparticles present in these materials may allow it to flow into dentinal tubules forming interlocking bonds and establishing a mineral infiltration zone with the posterior formation of hydroxyapatite [33]. Recent studies have demonstrated that Bio-C Sealer showed better cytocompatibility, mineralization capacity [20], higher penetration and better adaptation to the dentinal tubules [34] compared to other filling materials. Moreover, it was shown that teeth filled with calcium silicate-based sealers presented higher values for fracture resistance when compared to those filled with AH Plus [3, 15]. These findings contradict those described in our study, where teeth filled with Bio-C Sealer presented a similar mechanical behavior to AP groups. When compared to non-prepared teeth, filling with Bio-C sealer showed significantly worse fatigue behavior. This may be explained by the different methodologies applied since most studies regarding mechanical behavior of root filled teeth use static load tests [3, 15, 35]. One of the highlights of the present study was the use of a cyclic fatigue test to assess the mechanical behavior.

Tooth fracture is a consequence of cyclic fatigue that happens in the oral cavity in response to the stress caused during mastication. In this situation, failure happens in a much lower load than the load capacity [36]. The cyclic fatigue test used here simulates, under controlled parameters such as number of cycles, frequency, and load, the intermittent loading movements observed in the mouth [28]. Thus, cyclic fatigue tests provide results replicating the clinical conditions and the cyclic nature of chewing. Studies using static loading only provide the maximum critical stress through the increased applied load, which does not allow predicting failures over time, since they do not simulate the stimuli of the oral cavity [28, 36].

Due to its potential ability to bond to root dentin, the manufacturers claim calcium silicate-based sealers perform successfully regardless of using a main core material [19]. This kind of obturation would create a single interface between the root filling material and root dentin, forming a “monoblock” along the root canal walls, avoiding the creation of gaps between gutta-percha points and sealer [19]. In that regard, previous studies have reported that root canal sealers showed higher push-out bond strength values when the obturation was performed in bulk than when it was associated with a master gutta-percha point [6, 19, 24]. Despite these favorable findings, herein, the teeth filled without gutta-percha (AP-B and BC-B groups) showed a similar mechanical behavior compared to those with gutta-percha (AP-GP and BC-GP groups). Also, it is fundamental to consider the early failures evidenced in the groups without gutta-percha (BC-B and AP-B), starting to fail in steps 225 N (25,000 cycles) and 250 N (35,000 cycles), respectively. In contrast, the AP-GP groups started to present failures at approximately the double of the load 300 N (55,000 cycles).

Those early failures present in groups filled without gutta-percha could be justified by a thicker layer of sealer, which can shrink during setting and dissolve over time [24]. These findings contradict the possible advantage of the obturation in bulk once the absence of a main core material may compromise the spreading of the sealer along the root canal walls, facilitating the appearance of bubbles in the obturation mass. When subjected to cyclic loads, the presence of empty spaces in the root canal filling could induce the occurrence of early failures.
Regarding the fracture mode, fracture below the cement-enamel junction was the most prevalent pattern. The main reason for this occurrence might be the angle of the applied load, a factor that can influence fracture strength [37]. Anterior teeth are loaded in an unfavorable way during chewing and biting since the forces applied to them are oblique (not directed to their long axes) and consequently more harmful [37, 38]. According to our findings, the type of sealer and the filling technique had no impact on the fracture mode.

*In vitro* studies present inherent limitations; thus, the results of our study should be carefully analyzed. Human extracted teeth were used and we must assume that some anatomical variability will exist in this case. Another consideration is that periodontal ligament simulation was not performed in the present study. Some authors suggest this reproduction would affect the stress distribution during the fracture strength test [39]. However, there is a tendency not to perform the simulation in studies regarding fatigue resistance [28, 35, 40]. According to Marchionatti et al. [41], it may not be necessary to reproduce the periodontal ligament in those tests since it does not affect the fatigue resistance of teeth. Additionally, using an elastomeric material between the roots and the PVC cylinders could favor the displacement of the specimen, due to the direction of load application, impairing the test accomplishment.

## 5 Conclusion

When filling endodontically treated teeth, the use of calcium silicate-based sealer is valid, both as bulk or associated with gutta-percha as a main core material, as it resulted in similar mechanical performance to an epoxy resin-based sealer. Despite that, the use of gutta-percha, as a main core material, seems to reduce the risk of premature failures.

### Declarations

**Compliance with ethical standards:**

Support/Sponsorship:

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We emphasize that the aforementioned institutions did not have any role in the study design, data collection or analysis, the decision to publish, or in preparing the manuscript.

Conflict of Interest Statement:

The authors deny the existence of any conflict of interests.

Ethical approval:

This study received approval from the Research Ethics Committee at the Federal University of Santa Maria (certificate number: 30345120.7.0000.5346) in the city of Santa Maria, Brazil.

### References


Figures

Figure 1

Representative images of the failure modes: A – Mode I, fracture above the cement-enamel junction (reparable); B – Mode II, fracture below the cement-enamel junction (irreparable).