

ORIGINAL RESEARCH

Root-end resection with or without retrograde obturation after orthograde filling with two techniques: A micro-CT study

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Abstract

To evaluate the filling ability of two orthograde obturation techniques followed by the apical resection with or without retrograde obturation through micro-computed tomography (CT). Thirty-two single-rooted permanent teeth were prepared and randomised into four groups ($n = 8$) according to the orthograde obturation technique (single cone technique [SCT] and mineral trioxide aggregate placement) combined or not with retrograde obturation. The volume of voids (VoV) within the entire endodontic space, the apical 3 mm, and 1 mm after root resection was calculated by micro-CT. Statistical analysis showed no significant difference among the groups regarding the total VoV in all root canals, as well as within the apical 1 mm after root resection. The SCT and apical resection without retrograde filling showed significantly better results in terms of VoV at the apical 3 mm after root resection. Within the study limitations, SCT associated with apical resection without retrograde preparation exhibited a similar or less amount of voids than the other groups.

KEYWORDS

apical resection, micro-CT, mineral trioxide aggregate, retrograde filling, single cone technique

INTRODUCTION

Endodontic surgery aims to remove the residual micro-organisms inside the root canal and guarantee this result over time with a proper apical seal [1–3]. Several procedures must be carried out properly to render endodontic surgery effective such as the haemostasis, the detection and removal of the lesion, the retrograde cavity preparation, and the correct placement of the filling materials [1]. Techniques based on the 3 mm apical resection without retrograde preparation may represent a simplified and more

conservative alternative to the standard procedures of apical surgery [4]. Such simplification could be acceptable if there were suitable materials and techniques to overcome the drawbacks of their classic counterparts. Two possible options might be the intentional orthograde positioning of mineral trioxide aggregate (MTA) or the single cone technique (SCT) with bioceramic sealer [5, 6], followed by the apical resection without further retrograde steps. The SCT represents a further simplification being less operator sensitive and capable of high-quality sealing ability [7, 8]. The use of bioactive materials such as MTA allows a series

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of benefits including biocompatibility, dimensional and chemical–physical stability also after contamination with biological fluids, and most importantly the ability to stimulate the healing of the periapical tissues [8–10].

The purpose of this study was to evaluate the filling ability of two orthograde obturation techniques (SCT with bioceramic sealer and MTA placing) followed by the apical resection with or without retrograde obturation through micro-computed tomography (CT). The null hypothesis tested was there would be no difference between the two orthograde techniques associated or not to the retrograde obturation in terms of the filling ability both in the entire space and in the apical 3 and 1 mm of the resected tooth.

MATERIAL AND METHODS

Selection of teeth and sample preparation

Thirty-two single-rooted permanent human teeth were collected from a pool of teeth extracted for periodontal reasons. Based on a power analysis (G*Power 3.1.9.2 software, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), 8 samples/group were required to achieve a power of 80%. Through a radiographic analysis, the teeth presenting multiple canals, anatomical aberrations, restorations, root caries, root canal treatments, cracks, perforations, and canal curvatures greater than 10° [11] were excluded. Selected teeth were scanned by a micro-CT system (SkyScan 1072; Bruker microCT, Kontich, Belgium) with an isotropic voxel size of 30 µm to include samples with similar regular straight canals. The teeth were manually scaled to remove calculus remnants from their external root surfaces and stored inside sealed containers with buffered saline (PBS 3813; Sigma-Aldrich, St Louis, MO) at 37°C until use. A single expert operator performed all the operations to ensure standardised procedures for all experimental groups and guarantee a uniform comparison between the groups. The teeth were decoronated with a diamond disk (Keystone industries, Gibbstown, NJ, USA) to obtain roots with a standardised length of 12 mm. Working length (WL) was determined by introducing a size 10 K-file (MicroMega, Besançon, France) in the canal until it was visible at the apical foramen and subtracting 0.5 mm from this measurement.

Root canal preparation

The root canal instrumentation was performed using Hyflex EDM rotary nickel–titanium instruments (Coltene, Coltene/ Whaledent AG, Altstätten, Switzerland) up to size 40,0.04 taper in a closed root canal system. All rotary

files were used to instrument three canals and then replaced. Sodium hypochlorite 2.5% (3 ml) was used as an irrigant during the procedure. Then, all specimens received a final flush of 2 ml of distilled water (1 min), 2 ml 17% EDTA (1 min), 6 ml 3% NaOCl (3 min), followed by 2 ml of sterile saline solution (1 min). Finally, root canals were irrigated again with 2 ml distilled water and activated using ultrasonic files (three cycles of 20s each). After the final rinse, the canals were dried with ISO 40 paper points. All irrigating solutions were delivered with a 30 G Max-i-Probe irrigating needle (Dentsply Rinn, Elgin, IL, USA) placed 1 mm short of the WL.

Root canal filling and root-end treatment

The roots were randomised into 4 groups (n = 8) as follows:

- Group SCT– RF (SCT and apical resection without retrograde filling). Bioceramic sealer (BioRoot RCS, Septodont, Saint-Maur-des-Fossés, France) was mixed on a glass plate in the proportions indicated by the manufacturer (1 spoon of powder and 5 drops of liquid). Standardised volumes of sealer were measured in a Centrix syringe and then placed into the root canals using paper points to smear the canal walls; then gutta-percha cone size 40.04 (ROEKO EDM gutta-percha points, Coltene/Whaledent) was coated with the sealer and inserted to the WL [12]. The cone was cut at the orifice level with a hot instrument. After 2 months of storage in an incubator at 100% relative humidity at 37°C, the root tips (3 mm from the apex) were transversely sectioned at 90° to the longitudinal axis of the teeth with a carbide bur (HM 33IL-316-010, Meisinger, Neuss, Germany) under water cooling 3 mm from the anatomical apex. All apicectomy procedures were performed under a dental microscope (OPMI PICO; Carl Zeiss, Göttingen, Germany) at 10× magnification.
- Group SCT+ RF (SCT and apical resection with retrograde filling). The orthograde canal filling, storage, and the apical resection procedures were the same as SCT– RF group. A 3-mm-deep retrograde cavity was prepared with ultrasonic tips (AS3D, Satelec Acteon Group, Merignac, France) under continuous water spray and activated by a piezoelectric source (Newtron P5, Satelec Acteon) at a power setting of 6. To ensure uniformity of the root-end preparations, the operator made every effort to insert the tips approximately 3 mm into the canals and ensured a preparation diameter of 1.0 mm [13]. The cavities were dried with paper points and filled with progressive increments of MTA cement (ProRoot MTA, Dentsply Maillefer), which was mixed

and applied according to the manufacturers' instructions using a carrier system (MAP System, Dentsply Tulsa, Tulsa, OK, USA) and compacted with manual retrograde filling condensers.

- Group MTA– RF (orthograde MTA placement and apical resection without retrograde filling). The apical portion of the canal was filled with MTA cement (ProRoot MTA, Dentsply Maillefer) placed by the MAP System (Dentsply Tulsa) and compacted with previously calibrated manual pluggers. Following an incremental technique, the cement was compacted up to 6 mm from the working length. The empty canal portion was backfilled with thermoplasticized gutta-percha, which was injected (Obtura III Max System, Obtura, Algonquin, IL, USA) and condensed with manual pluggers. The root apices were sectioned as described in the SCT– RF group following the same storage protocol.
- Group MTA + RF (orthograde MTA placement and apical resection with retrograde filling). The orthograde canal filling and the apical resection were the same described for the MTA– RF group and the retrograde filling procedures were the same for the SCT + RF group.

Successively, periapical radiographs of all samples were taken to ensure the quality of retrograde filling and the samples were stored in an incubator (37°C, at 100% relative humidity) for 7 days [13].

Micro-CT evaluation

The samples were scanned at t_0 , after root canal orthograde obturation; and t_1 , after root apex resection procedures. Micro-CT scans of the samples were performed using a custom-made cone-beam system called TomoLab based on a microfocus source [14]. The cone-beam geometry permits achieving a spatial resolution close to the focal spot size [14]. The samples were placed on a dedicated round base and the acquisitions were performed according to the following parameters: a) source-sample distance (F_{OD}), 125 mm; b) source-detector distance (F_{DD}), 250 mm; magnification, 2×; start, 2 × 2; resolution, 12.5 μm; tomography resolution (pixels), 2004 × 1335; section size (pixels), 1336 × 1336; number of tomographs, 1440; number of sections, 1332; E = 80 kV, I = 200 μA; and exposure time, 2.5 s. The images were elaborated by commercial software (Cobra Exxim, Pleasanton, CA, USA) and the software Amira (Thermo Fisher Scientific, Waltham, USA) was used for the co-registration of the sample volumes. The segmentation of the materials used in the testing samples and the quantification of their volumes were performed by BoneJ plugins [15] implemented

with Fiji software [16]. The micro-CT analysis allowed to quantify the volume of voids (VoV) within the entire endodontic space, the apical 3 mm and the last 1 mm of the canal after root resection.

The analysis provided the following data (μm³):

1. total VoV;
2. the relative VoV at the dentin-material interfaces:
 - a. dentin-guttapercha interface;
 - b. dentin-sealer interface;
 - c. dentin-MTA interface;
3. the relative VoV at the material-material interfaces:
 - a. guttapercha-sealer interface;
 - b. guttapercha-MTA interface;
4. the relative VoV within the single materials:
 - a. gutta-percha;
 - b. sealer;
 - c. MTA.

The VoV in the resected teeth with or without retrograde obturation was calculated by subtracting the filling material volume ($Filling_{Vol}$) from the post-instrumentation root canal volume ($Canal_{Vol}$) cropping and excluding the resected root-end and applying the entire endodontic space or the last apical 3 mm or the last 1 mm as a region of interest: $VoV = Canal_{Vol} - Filling_{Vol}$. The percentage volume of voids (VoV%) was calculated as follows: $VoV\% = VoV / Canal_{Vol} \times 100$.

The volume of the gap between the tooth surface and the different orthograde or retrograde filling materials as well as the volume of voids in the different orthograde or retrograde filling materials were measured [17]. A grey scale ranging from 56 to 255 indicated the volume of the filling material ($Filling_{Vol}$), 0 to 10 indicated the volume of the voids between the different filling materials and the root canal wall (V_{out}), and 0 to 56 indicated the volume of voids within the single filling material (V_{in}). The relative percentage of VoV at the: dentin-material interfaces (a), material-material interfaces (b), and within the single materials (c) were calculated as follows:

- a. $\%V_{out} = V_{out} / Canal_{vol} \times 100$
- b. $\%V_{in} = V_{in} / Canal_{vol} \times 100\%$ $V_{imat} = VoV - V_{out} - V_{in} / Canal_{vol} \times 100$

Statistical analysis

For the scalar dependent variables, Shapiro–Wilk and Levene tests were used to assess the normality of the distribution and the equality of variances, respectively. In the considered canal portions, the differences among groups in terms of total VoV were evaluated using the

Kruskal–Wallis test. The Mann–Whitney test with Bonferroni correction served for the pairwise comparisons. The level of significance was set at 5% (Statistical Package for Social Sciences v.15, SPSS Inc., Chicago, IL, USA).

RESULTS

The preliminary analysis of root canal volume after preparation (at t_0) for canals root filled with SCT (10.26 mm^3) or orthograde MTA (10.79 mm^3) revealed no significant difference between them ($p > 0.05$). This result allowed a uniform comparison between the experimental groups.

Tables 1–3 show the overall and differentiated values of the percentages of VoV within the whole endodontic space, the apical 3 mm of the resected root, and the apical 1 mm of the resected root, respectively.

As a general trend, minimal voids formation was observed within the endodontic space in all groups (Tables 1 and 3). No significant difference was shown between the four groups in volume of voids considering the total endodontic space as well as the 1 mm of the canal after root resection ($p > 0.05$).

As for the volume within the apical 3 mm of the canal after root resection, the group SCT– RF (SCT and apical resection without retrograde filling) exhibited the least amount of voids compared to the other groups ($p < 0.05$) (Table 2). Moreover, a statistically significant difference was observed when the group SCT+ RF was compared to groups MTA– RF and MTA+ RF ($p < 0.05$), with no significant difference between MTA– RF and MTA+ RF ($p > 0.05$). More details on the 4 criteria are reported in

Tables 1–3 with the relative VoV at the dentin-material and material-material interfaces regrouped in the item “voids at the interfaces.”

The qualitative analysis of the three-dimensional reconstructions of the samples showed that the voids were mainly accumulated at the coronal level in the SCT groups (Figure 1a and b). For SCT groups, the analysis did not show voids in the gutta-percha portion. In MTA– RF, the voids were detected in most specimens within the MTA mass (Figure 1C). In MTA+ RF, the MTA had minimal internal voids, while slightly greater VoV was observed inside the injected gutta-percha mass (Figure 1D).

DISCUSSION

Despite some possible disadvantages such as the high cost and the lack of its applications in long-term studies, micro-CT is a noninvasive, nondestructive, and high-resolution technology, representing a reliable investigation tool in many different applications, including dentistry and especially endodontics [18]. Micro-CT ensures the three-dimensional study of the root canal system and the understanding of its influence on the different treatment/retreatment procedures, by reconstructing digital cross sections of the teeth [17, 19]. The digital acquisitions can be stacked to create 3D volumes providing reliable information for clinicians [20].

The MTA compaction was performed through calibrated manual pluggers. Some studies examined the efficacy of alternative MTA orthograde compaction techniques for filling the entire root canal volume. The

TABLE 1 Distribution of filling voids: Mean values and standard deviations in the entire endodontic space

	SCT– RF	SCT+ RF	MTA– RF	MTA+ RF
Total % of VoV	0.82 ^a ± 0.58%	1.88 ^a ± 1.49%	1.08 ^a ± 0.50%	1.16 ^a ± 0.50%
Voids at the interfaces				
Dentin—gutta-percha	0.00 ± 0.00%	0.00 ± 0.00%	0.05 ± 0.04%	0.27 ± 0.12%
Dentin—sealer	0.32 ± 0.23%	0.47 ± 0.46%	–	–
Dentin—MTA	–	0.01 ± 0.02%	0.00 ± 0.00%	0.00 ± 0.00%
Gutta-percha—sealer	0.47 ± 0.23%	1.02 ± 0.54%	–	–
Gutta-percha—MTA	–	0.26 ± 0.22%	0.39 ± 0.20%	0.08 ± 0.06%
Internal voids				
Gutta-percha	0.00 ± 0.00%	0.01 ± 0.02%	0.18 ± 0.06%	0.58 ± 0.16%
Sealer	0.04 ± 0.03%	0.03 ± 0.05%	–	–
MTA	–	0.08 ± 0.15%	0.45 ± 0.21%	0.15 ± 0.13%

Different superscript letters in the same row indicate a statistically significant difference between the total % of VoV of the four groups. The hyphen (–) indicates items not applicable to the group. ^arefer to the row of Total % Vov.

Abbreviations: MTA+ RF, Orthograde MTA placement and apical resection with retrograde filling; MTA– RF, Orthograde MTA placement and apical resection without retrograde filling; SCT+ RF, SCT and apical resection with retrograde filling; SCT– RF, SCT and apical resection without retrograde filling.

TABLE 2 Distribution of filling voids: Mean values and standard deviations in the apical 3 mm of the resected root

	SCT– RF	SCT+ RF	MTA– RF	MTA+ RF
Total % of VoV	0.06 ^a ± 0.10%	0.76 ^b ± 0.81%	2.06 ^b ± 1.92%	1.53 ^b ± 1.79%
Voids at the interfaces				
Dentin—gutta-percha	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.15 ± 0.25%
Dentin—sealer	0.03 ± 0.06%	0.00 ± 0.00%	–	–
Dentin—MTA	–	0.25 ± 0.41%	0.00 ± 0.01%	0.00 ± 0.00%
Gutta-percha—sealer	0.00 ± 0.00%	0.00 ± 0.00%	–	–
Gutta-percha—MTA	–	0.45 ± 0.37%	0.00 ± 0.00%	0.35 ± 0.72%
Internal voids				
Gutta-percha	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.24 ± 0.68%
Sealer	0.00 ± 0.00%	0.00 ± 0.00%	–	–
MTA	–	0.07 ± 0.12%	1.70 ± 0.78%	0.56 ± 0.70%

Note: Different superscript letters in the same row indicate a statistically significant difference between the total % of VoV of the four groups. The hyphen (–) indicates items not applicable to the group. ^{a, b} refer to the row of Total % VoV.

Abbreviations: MTA+ RF, Orthograde MTA placement and apical resection with retrograde filling; MTA– RF, Orthograde MTA placement and apical resection without retrograde filling; SCT+ RF, SCT and apical resection with retrograde filling; SCT– RF, SCT and apical resection without retrograde filling.

TABLE 3 Distribution of filling voids: Mean values and standard deviations in the apical 1 mm of resected root

	SCT– RF	SCT+ RF	MTA– RF	MTA+ RF
Total % of VoV	0.01 ^a ± 0.02%	0.04 ^a ± 0.11%	3.24 ^a ± 5.88%	2.61 ^a ± 6.39%
Voids at the interfaces				
Dentin—gutta-perca	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
Dentin—sealer	0.00 ± 0.00%	0.00 ± 0.00%	–	–
Dentin—MTA	–	0.01 ± 0.04%	0.00 ± 0.00%	0.00 ± 0.00%
Gutta-perca—sealer	0.00 ± 0.00%	0.00 ± 0.00%	–	–
Gutta-perca—MTA	–	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
Internal voids				
Gutta-perca	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%	0.00 ± 0.00%
Sealer	0.00 ± 0.00%	0.00 ± 0.00%	–	–
MTA	–	0.00 ± 0.00%	1.08 ± 3.04%	0.43 ± 2.61%

Note: Different superscript letters in the same row indicate a statistically significant difference between the total % of VoV of the four groups. The hyphen (–) indicates items not applicable to the group. ^a refer to the row of Total % VoV.

Abbreviations: MTA+ RF, Orthograde MTA placement and apical resection with retrograde filling; MTA– RF, Orthograde MTA placement and apical resection without retrograde filling; SCT+ RF, SCT and apical resection with retrograde filling; SCT– RF, SCT and apical resection without retrograde filling.

manual compaction performed by endodontic nickel-titanium pluggers combined with an indirect activation by use of ultrasonic inserts seems to represent a valid and better alternative technique according to Yeung et al. [21]. However, another micro-CT study seems to contradict the abovementioned hypothesis as reported that manual compaction allowed obtaining lower formation of voids compared to vibratory techniques with different durations [22].

Within the present outcomes, all four techniques guaranteed homogeneous root canal filling with the limited formation of voids. The two groups involving retrograde preparation and filling were used as a reference because

they are considered the gold standard in endodontic surgery [23, 24].

According to our results, regarding the volume of voids criterion, the group with SCT and apical resection without retrograde filling had a similar or less amount of voids than the other groups, in the entire space/1 or at 3 mm, respectively. Therefore, on the basis of the present outcomes, the null hypothesis is partially accepted. These results are probably related to the material characteristics and/or consistency [17]. Indeed, the handling and manipulation of MTA are often difficult for operators due to its granular consistency [25] and looseness [17], as well as the clinical difficulty to be easily carried and delivered,

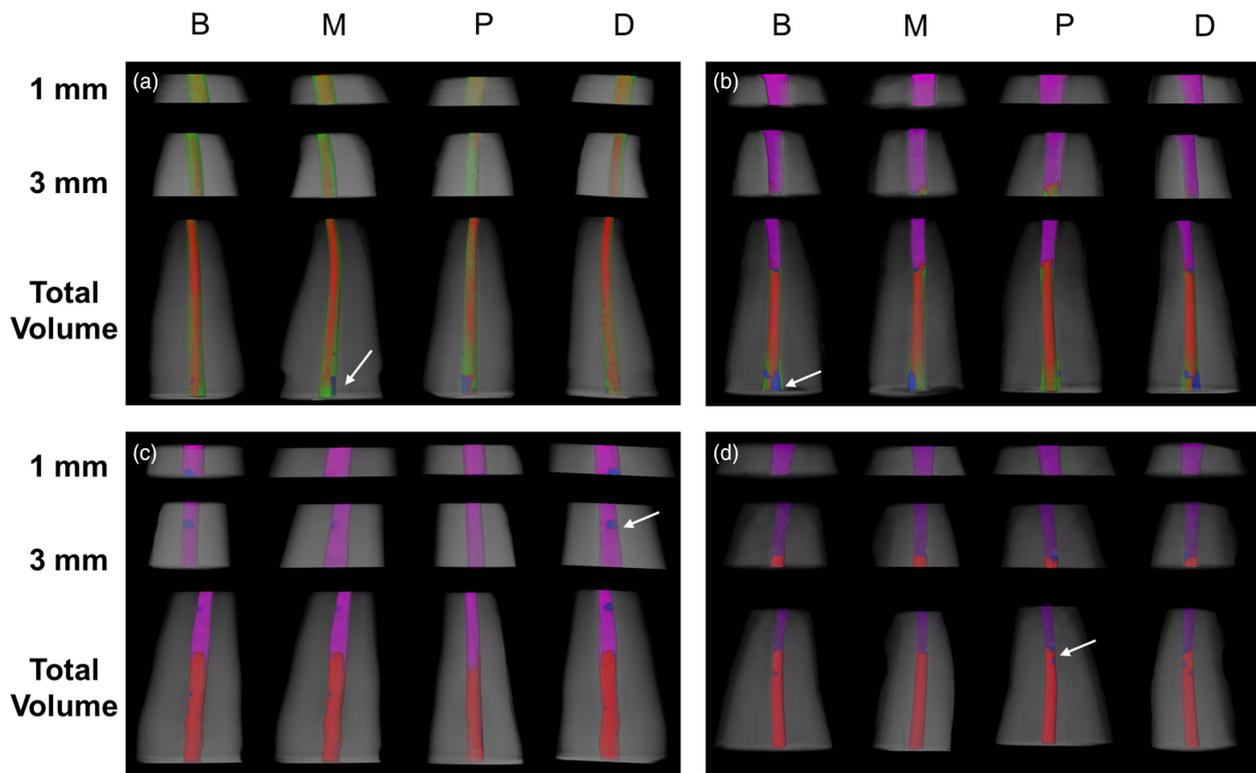


FIGURE 1 (a–d) representative 3D construction of the entire endodontic space as well as of the apical 3 and 1 mm of the resected root of group SCT– RF (single cone technique and apical resection without retrograde filling) (a); group SCT + RF (single cone technique with apical resection and traditional retrograde filling) (b); group MTA– RF (orthograde MTA placement and apical resection without retrograde filling) (c); group MTA + RF (orthograde MTA placement and apical resection with retrograde filling) (d). Green, endodontic sealer; red, gutta-percha; blue, voids; purple, MTA. The white arrows indicate examples of voids detected. B., Buccal; D, Distal; M, Mesial; P, Palatine

especially in the most apical part of the root canal [26]. On the other hand, the SCT associated with a bioceramic sealer is considered a simple, easy to learn, and replicable root filling method and therefore more clinically manageable [27]. Regarding the lack of difference between groups at 1 mm, it could be due to the reduced volume analysed at this level which may reduce the probability to detect voids.

As concerns for the criteria of voids at the interfaces and material, the voids were mainly accumulated at the coronal level in the SCT groups and at the interface of the filling materials with no voids detected in the gutta-percha portion probably due to the compact mass of single cone. In MTA– RF and MTA + RF groups, the voids were detected within the MTA mass and gutta-percha, especially in the last apical millimetres. These results could be attributed to the physical properties of materials used as well as to technical features of obturation techniques. Unlike the SCT, orthograde obturation by means of MTA and thermoplasticized injectable gutta-percha requires more increments and more clinical passages which could contribute to voids formation [28]. Moreover, the MTA viscosity and its grainy consistency could hinder the condensation of the MTA increments, particularly in the

apical region [17, 29]. The importance of accurate identification of the areas in which the voids are located – especially in secondary root canals – could have clinically relevant implications since voids represent potential areas for microorganisms proliferation [13, 30].

The study has some limitations. First, it was an ex vivo study and consequently, some conditions in vivo, including bleeding control and clinician experience, could influence the outcomes obtained. Furthermore, we included only single straight rooted permanent human teeth to guarantee the sample standardisation. Anyway, clinical variables such as the anatomic complexities, the type and tooth position, and number and form of roots, represent additional factors able to affect the quality of root-end filling [13].

Despite retrograde root canal filling being recommended to avoid any possible leakage due to undetected persistent residual bacterial infection [31], it does not seem to improve prognosis significantly compared to teeth with a dense orthograde root canal filling [32, 33]. Thus, the SCT associated with a bioceramic sealer could represent in some selected cases a promising alternative technique compared to the current gold standard for apicectomy in terms of clinical convenience. Further investigations

following consistent guidelines for this procedure are necessary to confirm these *in vitro* results.

Within the study limitations, SCT with a bioceramic sealer associated with apical resection without retrograde preparation had a similar or less amount of voids than the other groups, in the entire space/1 or at 3 mm, respectively. Minimal voids formation was observed within the endodontic space in all groups.

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AUTHOR CONTRIBUTIONS

D.A., E.P., M.D.B., and M.L. performed the research. D.A., E.P., M.D.B., and L.G. designed the research study. D.A., M.L., D.P., and L.B. contributed essential reagents or tools. D.P., G.R.M.L.R., L.B., L.G., and E.P. analysed the data. D.A., M.D.B., G.R.M.L.R., and E.P. wrote the paper. All authors have contributed significantly, and all authors are in agreement with the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration. The Research Ethics Committee has confirmed that no ethical approval is required.

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