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ORIGINAL ARTICLE

Calcium Silicate-based Intracanal Medication: Physicochemical Properties and Effectiveness of Techniques for Removing Medication from the Human Root Canal

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ABSTRACT

Objective: Our purpose was to investigate the physicochemical properties of Bio-C Temp (Angelus, Londrina, PR, Brazil), a bioceramic intracanal medication, and whether its residues remain adhered to dentine walls after conventional manual irrigation (CMI) or passive ultrasonic irrigation (PUI) in comparison to the Calen (SS. White, Rio de Janeiro, RJ, Brazil).

Methods: The pH after 12 hours, 1 day, 3 days, 7 days, 14 days, 21 days and 28 days and the flow, radiopacity, and solubility of the medications after immersion for 7 and 30 days in distilled water (dH_2O) or phosphate-buffered saline (PBS) solution were evaluated. Filling capacity and volumetric changes after 14 days were assessed by micro-computed tomography (micro-CT). The residues of medications after CMI or PUI were analysed with scanning electron microscopy. Statistical analysis was performed using ANOVA and Tukey's post-hoc test, Student's t-test, or the Kruskal-Wallis and Dunn post-hoc test (α=0.05).

Results: Bio-C Temp presented lower pH, flow, volumetric change, and weight loss after immersion in PBS on the 7th and 14th days ($p<0.05$) and greater radiopacity and filling capacity ($p<0.05$) than Calen. Both medications showed lower solubility in PBS than in dH_2O ($p<0.05$). There was no difference in the residue content of the two medications between the two irrigation methods in three-thirds of the roots ($p>0.05$).

Conclusion: Although Bio-C Temp had less volumetric loss and satisfactory filling capacity, this medication provided lower alkalinity than Calen. Furthermore, neither CMI nor PUI completely removed the medicament residues within the human root canal.

Keywords: Bioceramic intracanal medication, medication debris, micro-CT, radiopacity, volumetric change

HIGHLIGHTS

- Bio-C Temp presented satisfactory physicochemical properties; however, calcium hydroxide provides a microenvironment with a higher alkaline pH, a desirable property in intracanal medication.
- The CMI and PUI irrigation techniques were able to remove both intracanal medications, but neither was completely effective.
- Assessment of the physicochemical properties of endodontic materials is essential to ensure their clinical indication.

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INTRODUCTION

Calcium hydroxide-based pastes are the intracanal medication of choice for different endodontic conditions (1). Calcium hydroxide-based pastes are used to optimise root canal disinfection after biomechanical preparation,

with periodic changes being recommended in cases where there is a need for the formation and repair of mineralised tissue (2). In this way, calcium hydroxide-based intracanal medications are indicated for treating periapical lesions, root resorption, root perforation, apexification, and dental trauma and for use as pulp capping agents (3).

Calcium hydroxide stands out for its antimicrobial and biological properties (4) largely due to ionic dissociation that gives rise to calcium and hydroxyl ions, providing an alkaline microenvironment that favours the formation of mineralised tissue (3) and bacterial enzyme inhibition (1). Although calcium hydroxide-based medications have adequate biological properties (4, 5), long-term placement of the paste inside the root canal can affect the mechanical properties of root dentine (2), increasing susceptibility to fracture (6).

Calcium silicate-based (bioceramic) cements are hydraulic biomaterials widely used in endodontics mainly due to their bioactivity, that is, the ability to form an apatite structure in contact with body fluids (7). These materials have different consistencies according to their clinical application and are subdivided into root canal filling sealers and repair materials. Repair materials represent a regenerative approach for the pulp and periodontium and are indicated for treating teeth with incomplete rhizogenesis, root perforation, retrograde filling, direct capping and capping agents during pulpotomy (1). In addition to the use of repair cements, these materials have been developed for use as root canal filling sealers. Calcium silicate-based root canal filling sealers combined with gutta-percha cones are usually used to fill root canals, aiming at a sealing that favours the success of endodontic treatment (8, 9).

Calcium silicate-based biomaterials have emerged as candidates for regenerative endodontic treatment as coronal barriers, sealing the previously induced blood clot structure. The formation of blood clots in the apical third of the root promotes greater deposition of mineralised tissue, advancing traditional apexification procedures for treating immature permanent teeth (10). Regenerative endodontic procedures provide conditions for the continuity of apical formation, increased dentine wall thickness, and bone repair (11).

Considering the biocompatibility and bioactivity of bioceramic materials, the market has launched silicate-based intracanal medications (12). Bio-C Temp (Angelus, Londrina, PR, Brazil) was the first intracanal bioceramic medication based on calcium silicates, available in a ready-to-use syringe. According to the manufacturer, Bio-C Temp is indicated for endodontic treatment and retreatment in teeth with perforations and external or internal resorptions. This bioceramic medication does not require frequent changes since it exhibits low solubility (13).

Bio-C Temp showed noncytotoxic effects when diluted extracts were added to fibroblasts (14), human dental pulp cells (hDPCs) (15, 16) and Saos-2 cells (4), and it has been suggested that this medication has osteogenic potential (4). Furthermore, Bio-C Temp is biocompatible and does not promote changes in hepatic enzymes in rats following subcutaneous implantation (5). Although Bio-C Temp is radiopaque (14–16) and releases hydroxyl groups, providing an alkaline microenvironment (14, 16), there is evidence suggesting that this medication has low antibacterial and antibiofilm activity against Enterococcus faecalis (4). Moreover, Bio-C Temp caused coronary discolouration after 21 days of simulated root canal filling (11).

Studies evaluating the physicochemical properties are relevant for predicting the clinical behaviour of medications (17, 18) since the physicochemical features interfere with their effectiveness and, consequently, the prognosis of endodontic treatment (19). Comparing new medications with those considered the "gold standard" is essential for understanding their clinical behaviour (12). Evaluation of filling capacity and volume loss using micro-computed tomography (micro-CT) (20) may provide a better understanding of the properties of this intracanal medication since flaws in intracanal filling and high solubility of intracanal medications can lead to treatment failure (19, 21). Although intracanal medication is necessary in some circumstances, incomplete removal of medication can impair the adhesion of root canal filling sealers to the dentine. Therefore, this study aimed to evaluate the physicochemical properties, such as the pH, radiopacity, flow, solubility, volumetric change, and filling capacity, of Bio-C Temp in comparison with those of Calen (SS. White, Rio de Janeiro, RJ, Brazil), a calcium hydroxide-based intracanal medication. Moreover, the presence of medication residues adhering to the dentine walls of the root canal after two irrigation techniques was also evaluated. The null hypothesis was that there would be no difference between these two intracanal medications.

MATERIALS AND METHODS

Details such as the trade name, chemical composition and manufacturers can be found in Table 1.

pH

After the medication was inserted inside into polyethylene tubes (n=10 in each group) with standardised size (length of 10 mm and 1.6 mm internal diameter), the tubes filled with medications were placed into bottles containing 10 mL of distilled water (dH_2O) and stored at 37°C. Using a digital pH meter (Digimed, SP, Brazil), the pH of each sample was measured after 12 hours and 1, 7, 14, 21 and 28 days of immersion at 25°C. Bottles containing only dH_2O were used as controls. Each sample was analysed in triplicate, and the mean pH was obtained (22).

TABLE 1. Chemical composition and manufacturers of Bio-C Temp and Calen intracanal medications

Intracanal medication	Composition	Manufacturer
BIO-C TEMP	Calcium silicates, calcium aluminate, calcium oxide, calcium tungstate, titanium oxide, dispersing agent	Angelus, Londrina, PR, Brazil
Calen	Calcium hydroxide, zinc oxide, polyethylene glycol 400	SS. White, Rio de Janeiro, RJ, Brazil

Radiopacity

Acrylic resin moulds with cavities 10 mm in diameter and 1 mm deep were filled with Calen or Bio-C Temp (n = 6 in each group). The moulds filled with medications and an aluminium scale with variable thicknesses (2 to 16 mm, in 2 mm increments) were positioned on occlusal radiographic films (Kodak Comp, NY, USA). A GE 1000 X-ray unit (X-ray Appliance GE 1000 - General Electric, WI, USA) adjusted at 60 kV, 7 mA, and 0.32 pulses per second with a focus-film distance of 33 cm was used. The films were processed, digitized and evaluated using UTHSCSA ImageTool for Windows version 3.00 software to determine the radiopacity equivalence of the medications in millimetres of aluminium (22).

Solubility

Polyethylene tubes (n = 6 in each group) were filled with Bio-C Temp or Calen and stored at 37°C. After 24 hours, the samples were weighed using an analytical balance (OHAUS Adventurer®, Nova Jersey, USA). The tubes were placed in bottles with 7.5 mL of dH_2O and maintained at 37°C for 7 or 14 days (6 samples of each group and period). Afterwards, the tubes were kept in a dehumidifier and weighed until they reached mass stability. Mass loss was calculated as the difference between the initial and final masses, and the value was expressed as a percentage (22). This analysis was also performed by immersing polyethylene tubes filled with Bio-C Temp or Calen in phosphate-buffered saline solution (PBS), as described by Torres et al. (20).

Flow

Flow analysis was conducted as recommended by the International Standard Organization 6876:2012 (23). Using a graduated syringe, 0.05 mL of Bio-C Temp or Calen was placed on a glass plate (n =10 in each group). Then, another glass plate (20 g) was placed on top of the plate, and a weight of 100 g was added. After 10 minutes, the maximum and minimum diameters of the paste were measured. In the case of a difference between diameters of less than 1 mm, the mean was used for the assay (22).

Volumetric Change and Filling Capacity

The protocol used in this study to obtain human teeth was approved by the Research Ethics Committee of the Araraquara Dental School of Dentistry, State University of São Paulo-UNESP, Brazil (CAAE: 11714518.3.0000.5416), and was conducted in accordance with the World Medical Association's Declaration of Helsinki. This research was carried out using extracted human teeth, which were obtained from the tooth bank of the Araraquara Dental School. The teeth were scraped off tissue residue, disinfected in 2.5% NaOCl for 5 min, and maintained at 4°C in 0.1% aqueous thymol solution.

The sample size for these assays was calculated with G*Power 3.1.7 for Windows program (Heinrich Heine University, Germany). ANOVA was performed considering an alpha-type error of 0.05 and a beta power of 0.95 for the variables. As previously described (20, 23, 24), the sample size needed was 4 teeth per group. Therefore, considering the risk of tooth loss during the methodology, a sample of 6 teeth per group was estimated.

Twelve human mandibular premolars were selected after radiographs. Teeth with apical curvatures, two root canals, treated canals, external or internal root resorption and root canals with a diameter greater than a manual ISO #10 instrument were excluded. After crown removal, the root length was standardised to 15 mm, and the tooth's working length (WL) was standardised to 1 mm short of the major apical foramen. Initially, the glide path was determined with a K#10-type file instrument (Dentsply Sirona, Switzerland). Root canals were prepared with Easy ProDesign Logic instruments (Easy Equipamentos Odontológicos, MG, Brazil) of 25/.01, 25/.06 and 40/.04 attached to an electric motor (VDW.SILVER, VDW, Germany), up to the WL (25). Throughout the preparation, the canals were flooded with an irrigating solution and at each instrument change, the canals were irrigated, totalling 5 mL of 2.5% sodium hypochlorite (NaOCl). After the end of instrumentation, the canals were washed with 5 mL of 2.5% NaOCl, followed by 2 mL of 17% ethylenediaminetetraacetic acid (EDTA; Biodinâmica, PR, Brazil) for 3 minutes. The canals were then irrigated with 5 mL of physiological solution, and sterile absorbent paper tips were used to dry the root canals.

Initial scanning by micro-CT (SkyScan 1176. Bruker, Kontich, Belgium) was used to standardise the Bio-C Temp and Calen groups according to the average value of the root canal volume. The first scan was performed with the following parameters: 80 kV voltage, 300 mA current, 35 µm voxel size, a copper and aluminium (Cu + Al) filter, and 360° rotation. The volume of the root canal of each tooth was calculated, and with stratified random sampling (26), the teeth were divided into 2 groups according to the medication used (n = 6 samples in each group). Calen paste was inserted into the root canal using an endodontic syringe attached to a Septoject XL needle (Septodont Brazil Ltda., SP, Brazil) positioned 2 mm short of the WL. Bio-C Temp was brought into the canal using the applicator tip provided by the manufacturer, which was inserted near the root apex. Then, the roots were radiographed to confirm the filling, and the temporary sealing was performed.

Immediately after filling the canals, the roots were scanned. The roots were also scanned after 14 days of immersion in PBS using a 9 µm voxel size. NRecon software (V1.6.10.4, Bruker-MicroCT) was used to reconstruct the images after determining the following parameters for each medication: smoothing, beam hardening and ring artefacts. The reconstructed images were superimposed on the different time points for geometric alignment using Dataviewer software (V1.5.2.4; Bruker). Quantitative analysis of 3D root canal images was performed with CTAn software (V1.15.4.0; Bruker-micro-CT). The filling capacity (%) of intracanal medications was calculated from the volume occupied by Bio-C Temp or Calen paste in the total volume of the root canal, as obtained by the initial micro-CT scans. Volumetric change (%) was calculated by the following formula [volumetric change = (final volume x 100/initial volume) - 100], where the final volume is that obtained after 14 days of immersion in PBS and the initial volume represents the value occupied by medication immediately after filling of the root canal (27). 3D images were obtained with CTVox software (v.3.2, Bruker-microCT).

Analysis of Root Canals After Medication Removal

To evaluate medication removal, forty-eight human mandibular incisors obtained from the tooth bank of FOAr were disinfected with 2.5% NaOCl and stored at 4°C in a 0.1% aqueous solution of thymol. The incisors were selected and prepared in the same way as previously described. After biomechanical preparation, the medications were inserted into the root canals (n=24 samples in each group), and the roots were radiographed and sealed with a cotton pellet and Coltosol (Coltène, WhaleDent, Switzerland). The specimens were placed in an oven at 37°C and 95% humidity for 14 days. After this period, the temporary seal was removed, a 40/.04 instrument on the WL was used, and the groups were randomly distributed into 2 subgroups according to the final irrigation method employed (n=12 samples in each group): conventional manual irrigation (CMI) and passive ultrasonic irrigation (PUI). The solution volume was standardised in both techniques: 4 mL of 2.5% NaOCl, 2 mL of EDTA, and 5 mL of dH_2O (26). CMI was performed using a syringe (Ultradent Products, South Jordan, UT) with 21 mm Navitip 30 G needle without a bevel at 2 mm from the WL, with in-and-out movements, continuous flow and concomitant aspiration using the following solutions: 2 mL of 2.5% NaOCl, 2 mL of 17% EDTA and, subsequently, 2 mL of 2.5% NaOCl under agitation, using a #40 K-file instrument (Dentsply Sirona, Ballaigues, Switzerland) for 3 minutes. Then, 5 mL of dH_2O was used for the final irrigation of all the root canals.

PUI was performed with an E1 – Irrisonic ultrasonic insert (Helse Ultrasonic, SP, Brazil) positioned 2 mm short of the WL. The insert was activated by the ultrasonic device Ultrawave XS (Ultradent, South Jordan, USA) at 50 Hz frequency and 10% power, with entry and exit movements avoiding contact with the insert with the root canal walls. PUI was performed in three cycles of 20 seconds each with 2 mL of 2.5% NaOCl in the first and third cycles and 2 mL of 17% EDTA in the second cycle. A final irrigation of each sample was performed with 5 mL of dH_2O (26).

Then, the root canal was sealed with a cotton pellet, and grooves were made on the buccal and lingual surfaces of the teeth with a diamond disc. The roots were sectioned along the longitudinal axis with a surgical chisel (SSWhite/Duflex, RJ, Brazil). From the apical foramen, the roots were sectioned at 4 mm (apical third), 8 mm (middle third) and 12 mm (cervical third). Samples were prepared for analysis by scanning electron microscopy (SEM) at 20 kV (JSM-6610LV, JEOL, Tokyo, Japan). The amount of medication debris was estimated using representative SEM micrographs taken at 500× magnification, following a previously established scoring method (28):

1. The root canal wall is clean, exhibiting few particles of debris;
2. Debris forms small, sparsely distributed clusters;
3. Clumps of debris are often observed but occupy less than 50% of the root canal wall;
4. More than 50% of the root canal surface contains debris; and
5. Debris is present on almost the entire surface of the root canal wall.

Statistical Analysis

The data were analysed with GraphPad Prism 9.02 (GraphPad Software, Inc., CA, USA) statistical software (α=0.05). The pH and solubility values were analysed by one-way ANOVA and post hoc Tukey's test. Radiopacity, flow, volumetric change and filling capacity values were analysed using the Student's t-test. The Shapiro-Wilk normality test revealed that the data were normally distributed. Since the scores obtained after removing Bio-C Temp and Calen paste had a nonnormal distribution, they were analysed with nonparametric tests. For comparisons between medications in the same root third, the data were analysed using the Kruskal-Wallis test and Dunn test. The Friedman test was used to compare the thirds for each medication.

RESULTS

Micro-CT images showed that both medications had volume loss after 14 days of immersion in PBS (Fig. 1a) and were not able to completely fill the root canals (Fig. 1b). According to Table 2, Bio-C Temp had greater radiopacity ($p<0.0001$) and filling capacity ($p=0.0327$) than Calen paste. The greatest values of volume loss ($p=0.0181$) and flow ($p=0.0030$) were observed for Calen.

In all periods, the pH of Bio-C Temp was significantly lower than that of Calen (Table 3). After 24 hours, the medium with Bio-C Temp exhibited pH values greater than 10.0, whereas from 3 to 28 days, the solutions presented pH values above 9.

As shown in Table 4, both intracanal medications exhibited mass loss after immersion in dH_2O or PBS for 7 or 14 days. Bio-C Temp had a lower mass loss than Calen ($p<0.05$), except after immersion in dH_2O for 7 days when a significant difference was not detected between these medications. The mass loss of Bio-C Temp was significantly lower when immersed in PBS than in dH_2O at 7 and 14 days. For Calen, a lower mass loss in PBS medium than in dH_2O was observed only at 14 days ($p<0.05$).

Regarding residues (Fig. 2a-l and Table 5), no significant difference was detected between Bio-C Temp and Calen for both irrigation methods in the three root thirds. Significant differences between CMI and PUI were not detected in the cervical and middle thirds. In the apical third, the amount of residue of Bio-C Temp and Calen adhered to the dentine walls was significantly lower in the specimens cleaned with PUI than in those cleaned with the CMI method. For Bio-C Temp, no significant difference was detected among the thirds, regardless of the irrigation technique used.

DISCUSSION

This study evaluated the physicochemical properties and residues on dentine walls after removal of Bio-C Temp, using CMI and PUI, compared with a calcium hydroxide-based medication. The null hypothesis was not accepted because of significant differences between the medications.

Calen provided the highest pH to the medium in all periods, which remained almost unchanged for up to 28 days. A study comparing the pH of Bio-C Temp with that of Ultracal XS (UltraDent Product, Inc., SP, Brazil), a calcium hydroxide-based

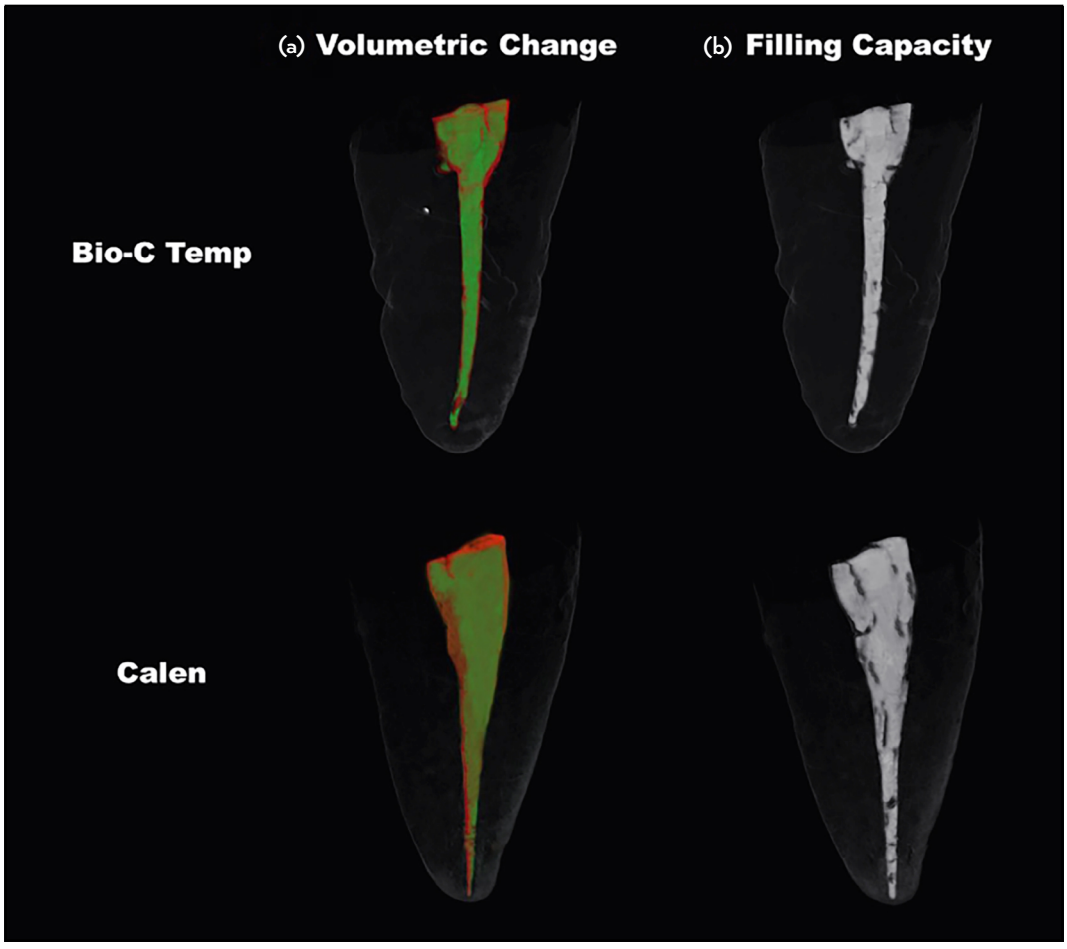

Figure 1. Three-dimensional reconstructions of mandibular premolars filled with Bio-C Temp and Calen. (a) Overlaying the volume of Bio-C Temp and Calen before (red) and after (green) immersion in PBS for 14 days. The red areas represent volume loss. (b) The fill ability of Bio-C Temp or Calen. The medications are in grey, and the failures are in black

PBS: phosphate-buffered saline

medication, also demonstrated greater alkalinity for calcium hydroxide paste after 1, 24 and 72 hours (14). This difference can be explained by the continuous release of hydroxyl ions that maintain elevated pH in the medium over time (29).

Although Bio-C Temp in contact with tissue fluids and water gives rise to calcium silicate hydrate and calcium hydroxide (14), which also dissociates into hydroxyl ions, favouring alkalinisation of the medium, the amount of calcium hydroxide available may be lower than that provided by Calen and Ultracal XS (4). It is also possible that the reduced amount of hydroxyl ion found in the medium containing Bio-C Temp may be explained by its low solubility compared to that of Calen. The low solubility of Bio-C Temp, as shown in the present study, may be responsible for the reduced volumetric change of this medication compared to that of Calen.

The released hydroxyl ions are responsible for a significant increase in tissue pH, making the environment unsuitable for bacterial growth. A high pH induces the inactivation of bacterial enzymes and changes the integrity of the bacterial membrane. E. faecalis are gram-positive bacteria that tolerate a high pH, around 9 to 11 (3) and are responsible for persistent root canal infection and are often associated with endodontic treatment failure. Therefore, calcium hydroxide pastes are considered suitable intracanal medications for teeth with pulp necrosis and apical periodontitis since these medications provide a high pH (approximately 12.6) to the microenvironment (1). Furthermore, the alkaline pH stimulates the deposition of mineralised tissue (3).

Bioceramic materials have different chemical components, and manufacturers do not often describe their formulation, particularly shortly after their launch. The composition difference, including component proportions, interferes with these materials' physicochemical and biological properties and clinical performance (17). Despite knowing the chemical composition of Bio-C Temp, its components' proportions are unknown. Different percentages of calcium silicates and calcium aluminates have been associated with lower calcium release and alkalizing activity of bioceramic materials (30).

Here, the solubility of the medications was evaluated after immersion in dH_2O and PBS, as there is a consensus that PBS is a medium that better mimics the physiological conditions (20). Our findings clearly showed that immersion in different

TABLE 2. Radiopacity (mm Al), flow (mm), volumetric change (%) and filling capacity (%) of Bio-C Temp and Calen medications (mean and standard deviation)

Test	Bio-C temp	Calen
Radiopacity (mm Al)	2.84±0.11[a]	1.78±0.45[b]
Filling capacity (%)	92.35±1.07[a]	88.41±1.28[b]
Flow (mm)	19.29±1.48[b]	23.43±0.28[a]
Volumetric change (%)	-27.12±2.12[b]	-34.38±1.59[a]

Different letters on the same line represent significant differences between intracanal medications (p<0.05)

TABLE 3. pH values (mean and standard deviation) of Bio-C Temp, Calen and control at each time point

Periods	Bio-C temp	Calen	Control
12 h	10.35±0.19[a]	11.41±0.10[b]	6.62±0.10[c]
1 days	10.11±0.16[a]	11.07±0.11[b]	6.69±0.17[c]
3 days	9.87±0.87[a]	11.40±0.74[b]	6.56±0.14[c]
7 days	9.86±0.95[a]	11.45±0.66[b]	6.48±0.41[c]
14 days	9.40 ±0.78[a]	11.49±0.34[b]	6.28±0.14[c]
21 days	9.32 ±1.13[a]	11.30±0.75[b]	6.33±0.23[c]
28 days	9.59±0.48[a]	11.48 ±1.07[b]	6.26± 0.18[c]

Different superscript letters represent significant differences among groups in the same period (p<0.05)

solutions interferes with the solubility of intracanal medications since Bio-C Temp and Calen, when immersed in dH_2O, presented greater mass loss than when immersed in PBS. It is conceivable that the hydrophilic properties of calcium hydroxide-based materials may explain the high solubility of Calen (21), as observed here. It has been suggested that the reduction in the solubility of calcium silicate-based materials when immersed in PBS (20, 31) could be explained by hydroxyapatite formation, which seems to prevent a further increase in solubility (31). In contrast, calcium hydroxide-based paste may undergo hygroscopic action, increasing weight when immersed in dH_2O.

In the present study, due to the absence of standards for intracanal medication evaluation, the solubility assay was conducted in accordance with ISO 6876:2012 (23), which establishes standards for the evaluation of endodontic cements. Although studies have evaluated the solubility of calcium hydroxide-based pastes after immersion in dH_2O (19, 21), it has been demonstrated that the immersion medium can affect the properties of bioceramic cements, such as the solubility and volumetric changes (20), which was also observed in the present study of both Calen and Bio-C Temp intracanal medications. In addition to the conventional assay, in the present study, micro-CT was also used to analyse the interface between dentine and material, which provides a three-dimensional evaluation (20, 32).

Here, micro-CT analysis revealed that Calen paste inside root canals presented great volumetric changes, culminating in significant mass loss due to its solubility. Micro-CT has been considered a useful tool for investigating endodontic materials' filling capacity and structural changes (32). This analysis is important because the volumetric loss of the material and the presence of voids after filling the root canals could prevent direct contact of the material with bacteria, impairing the antimicrobial action of the intracanal medication.

TABLE 4. Values (mean and standard deviation) of mass loss (%) of Bio-C Temp and Calen after immersion in distilled water and PBS

Immersion medium and time points	Bio-C temp	Calen
Distilled water - 7 days	14.87±1.79[Aa]	17.96± 2.44[Ba]
PBS - 7 days	9.72±0.93[Bb]	14.22±2.38[Ba]
Distilled water - 14 days	17. 23±1.02[Ab]	22.00±3.07[Aa]
PBS - 14 days	11.53±1.22[Bb]	15.56±1.54[Ba]

Different capital letters in the same column represent significant differences detected in the intracanal medication after immersion in different solutions for 7 and 14 days. Different lowercase letters in the line represent significant differences between intracanal medications in the same period (p<0.05). PBS: phosphate-buffered saline

TABLE 5. Median, minimum, maximum and first and third quartiles of residues of Bio-C Temp (BIO) and Calen (CAL) medication after conventional manual irrigation (CMI) and passive ultrasonic irrigation (PUI)

Root third	Group	Median	Min	Max	1st-3rd quartiles
Cervical	CAL/CMI[a,1]	1.9	1	4	1–3
	CAL/PUI[a,1]	1.5	1	2	1–2
	BIO/CMI[a,1]	2.5	1	4	1–4
	BIO/PUI[a,1]	1.9	1	3	1–2.75
Middle	CAL/CMI[a,1]	1.8	1	5	1–2
	CAL/PUI[a,1]	1.4	1	2	1–2
	BIO/CMI[a,1]	2.6	1	4	1.25–4
	BIO/PUI[a,1]	1.5	1	4	1–2
Apical	CAL/CMI[a,2]	3.8	1	5	2.25–5
	CAL/PUI[b,1]	1.6	1	3	1–2
	BIO/CMI[a,1]	3.1	1	4	2.25–4
	BIO/PUI[b,1]	1.5	1	4	1–2

Different letters represent significant differences between groups in the same root third. Different numbers represent significant differences among the root thirds of the same group (p<0.05). Min: Minimum, max: Maximum

In this context, despite the hydroxide-based paste showing greater flow than Bio-C Temp, it showed less filling capacity. Volumetric loss may be directly associated with the marked quantity of ions released to the microenvironment (33). Since the release of these ions is essential for the bioactivity and antimicrobial activity of intracanal medications, there must be a balance between minimal volume loss and sufficient ion release to maintain the biological properties of these medications (4).

In the present study, radiopacity evaluation was based on ISO 6876:2012 (23), which was developed for root canal sealers since there is no ISO standard for evaluating the radiopacity of intracanal medications. However, using an intracanal

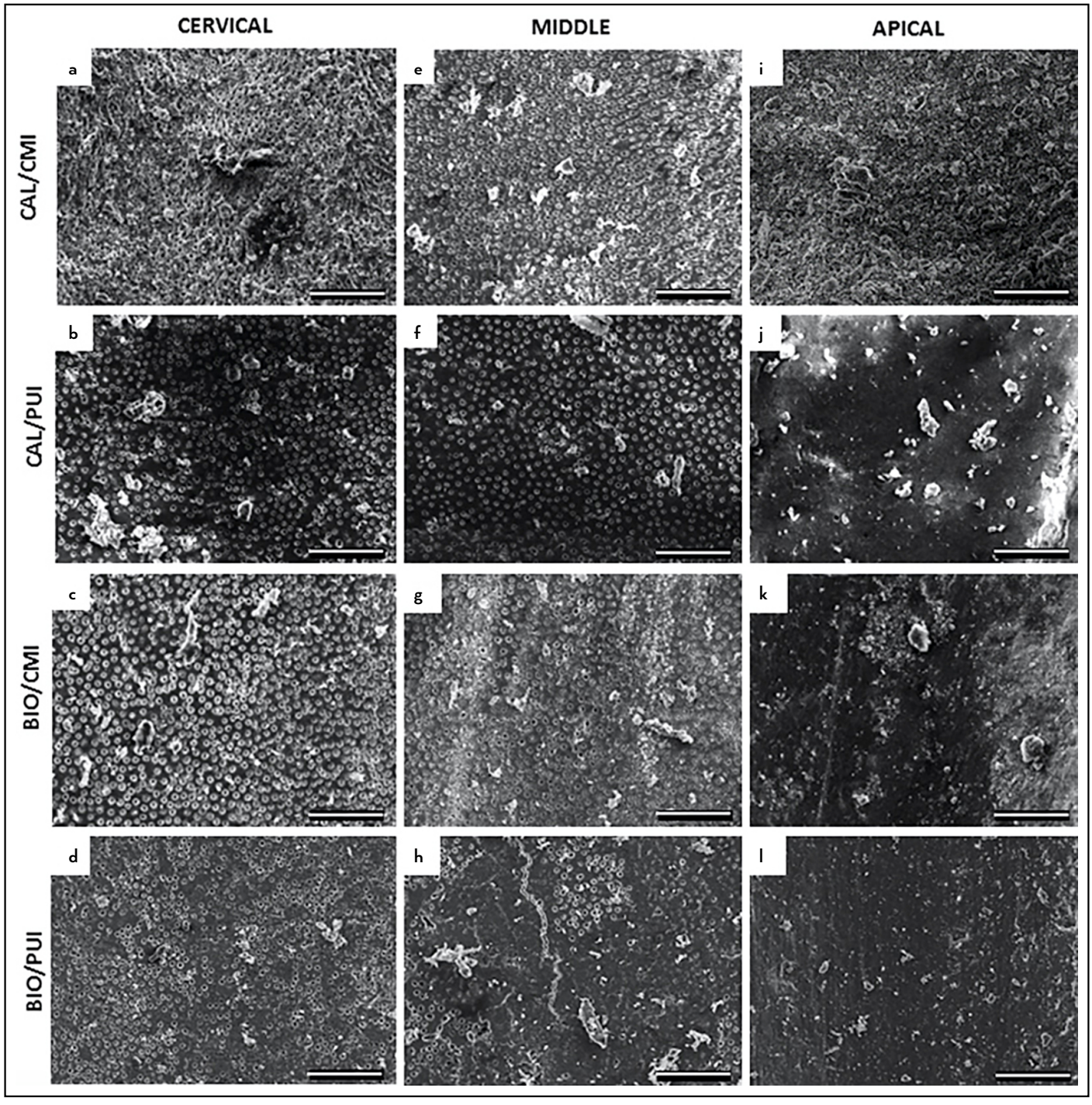

Figure 2. Electron micrographs obtained with SEM. Representative images showing portions of root canals after removal of Calen (CAL) and Bio-C Temp (BIO) using conventional manual irrigation (CMI) and passive ultrasonic irrigation (PUI) in the cervical (a-d), middle (e-h) and apical (i-l) root thirds. Bar: 52 µm

SEM: Scanning electron microscope

medication exhibiting radiopacity is essential for better visualization of the filling of the root canal system (34). Here, Bio-C Temp showed greater radiopacity than Calen, corroborating the findings of other studies. This difference can be explained by calcium tungstate and titanium oxide in the composition of Bio-C Temp (14, 15), while the Calen paste contains only zinc oxide as a radiopacifying agent. However, both medications had a radiopacity inferior to 3 mm Al, the minimum value for endodontic sealers (23).

Using PUI can enhance the cleaning effects by cavitation and acoustic microflow, allowing irrigation solutions to easily penetrate the working length and irregularities (35) and providing better removal of debris (36). PUI proved more effective than CMI in cleaning Bio-Temp and Calen in the apical third of the root canals. Using energy dispersive spectroscopy (SEM-EDS), different chemical elements of Bio-C Temp and Ultracal XS were observed adhering to the dentine surface of the root canals (37). Here, ultrastructural analysis revealed that neither the CMI

nor PUI cleaning techniques were able to completely remove intracanal medication residues from Bio-C Temp and Calen paste, supporting the concept that material residues can remain adhered to dentine walls despite the use of different irrigation techniques (38–40). Paste residues may persist on dentine walls, interfering with the adhesion of the root canal filling material and affecting the sealing capacity of the root canal sealers (38).

One of the limitations of this study is that there are no international standards for evaluating intracanal medication. Therefore, the methodology was based on standards recommended for endodontic cements. Although the results of the present study contribute to a better understanding of the characteristics of bioceramic intracanal medications, it should not be forgotten that in clinical use, this material comes into contact with inflamed periapical tissue. It is known that inflamed tissue exhibits, in addition to a low pH, different cell types and cytokines, constituting a microenvironment with conditions different from those evaluated in *ex vivo* models.

Our findings revealed that Bio-C Temp exhibits important physicochemical properties, such as a better filling capacity and radiopacity and lower volume loss and volumetric changes than Calen. In addition, the alkaline pH provided by Bio-C Temp to the microenvironment was lower than that of Calen, which may reduce its antimicrobial action, a desirable property in intracanal medication (4). Therefore, further studies are needed to evaluate the effectiveness of antimicrobials, their ability to induce bone repair, and the clinical behaviour of bioceramic-based intracanal medication.

CONCLUSION

Despite the reduced volumetric loss and satisfactory filling capacity of bioceramic medication, Bio-C Temp promoted lower alkalinity in the medium than Calen. Neither CMI nor PUI completely removed Bio-C Temp or Calen from the root canals, although the PUI technique was more effective than the CMI technique in cleaning the apical third of root canals.

Disclosures

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