Intracanal Antibacterial Effects of a Bioceramic Medication Compared With Calcium Hydroxide Pastes in Different Vehicles

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ABSTRACT

Introduction: The intracanal antibacterial effectiveness of a bioceramic medication was compared with calcium hydroxide pastes in different vehicles. **Methods:** Extracted mandibular incisors with a single long oval canal were selected and distributed into 5 groups based on anatomically paired microcomputed tomographic analyses. The root canals were prepared up to an instrument size 35/04 and contaminated for 30 days with a mixed bacterial culture from subgingival biofilm added with *Enterococcus faecalis*. The canals were medicated with Bio-C Temp (Angelus Indústria de Produtos Odontológicos, Londrina, PR, Brazil) or a calcium hydroxide paste in glycerin, camphorated paramonochlorophenol and glycerin (CHPG), or 2% chlorhexidine (CHCX). Control specimens were filled with sterile saline. After 7 days, the medication was removed by using rotary instruments and saline irrigation. A supplementary approach was conducted, which consisted of 2.5% sodium hypochlorite irrigation and agitation with XP-endo Finisher. Bacteriological samples were taken before application (S1) and after removal (S2) of the intracanal medication, and after the supplementary approach (S3). Bacterial DNA extracted from the samples was quantified using real-time polymerase chain reaction. **Results:** Bacterial counts were significantly reduced from S1 to S2 in all groups, except for the control group. As for S1-to-S3 changes, all groups (control included) showed significant bacterial reduction. S2-to-S3 changes were only significant in the CHPG and CHCX groups ($P < .05$). Intergroup comparisons showed no significant differences in S2 ($P > .05$), with all medications significantly better than the control ($P < .05$). In S3, only the CHPG and CHCX groups showed further significant bacterial reductions. Categorical data analysis showed no intergroup differences ($P > .05$). **Conclusions:** All tested medications significantly reduced the intracanal bacterial counts, with no significant differences between them. Final sodium hypochlorite irrigation and agitation using XP-endo Finisher further enhanced disinfection in the CHCX and CHPG groups. *(J Endod 2025;51:207–212.)*

KEY WORDS

Bioceramic material; calcium hydroxide; calcium silicate; intracanal medication; root canal treatment

SIGNIFICANCE

This study demonstrated that calcium silicate-based intracanal medication (Bio-C Temp) and calcium hydroxide intracanal pastes effectively reduced bacterial counts in root canals, with no significant differences between them. The supplementary approach with XP-endo Finisher to agitate the NaOCl improved disinfection of both CHCX and CHPG groups.

Although chemomechanical preparation plays a significant role in reducing bacterial counts in infected root canals, studies have shown that a large number of cases still harbor residual bacteria after preparation procedures[1-5]. This is conceivably because the main effects of chemomechanical procedures are mostly restricted to the main canal lumen and its immediate vicinity, but even in those areas, bacteria may still remain unaffected on some unprepared walls and recesses[6,7]. Because bacterial presence in the canal at the time of filling is an important risk factor for persistent apical periodontitis[8,9], there is a need to enhance disinfection after preparation. Intracanal medication is one of the most commonly recommended approaches to supplement bacterial elimination after preparation[10].

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Calcium hydroxide is the most widely used interappointment medication in endodontics, primarily because of its antimicrobial effects[11]. Because of its low solubility, the pH-dependent antimicrobial activity in the canal can be limited, especially because of the buffering ability of dentin and proteins from tissue remnants and bacteria[12,13]. To overcome these limitations, calcium hydroxide has been combined with other antimicrobial agents, such as chlorhexidine and camphorated paramonochlorophenol, with the purpose to improve and expedite bacterial killing, and expand the spectrum of activity[14-19].

Also considering calcium hydroxide limitations, alternative intracanal medications have been proposed. Bio-C Temp (Angelus Indústria de Produtos Odontológicos, Londrina, PR, Brazil) is a ready-to-use bioactive bioceramic medication, which contains calcium silicates associated with calcium tungstate, titanium oxide, calcium aluminate, calcium oxide, and a base resin that does not permit setting of the material. According to the manufacturer, Bio-C Temp does not require multiple changes, which should be an advantage when compared to calcium hydroxide pastes in inert vehicles. This bioceramic medication undergoes a hydration reaction that leads to the formation of calcium silicate gel and calcium hydroxide, the latter being claimed to be responsible for its antimicrobial action. Only a few studies evaluate the antibacterial effects of this bioceramic intracanal medication (Guerreiro et al., 2021; Patri et al., 2024), none comparing it to calcium hydroxide mixed with different vehicles. Therefore, this *ex vivo* study was undertaken to compare the intracanal antibacterial effectiveness of a bioceramic intracanal medication and calcium hydroxide pastes in different vehicles.

MATERIAL AND METHODS

Specimen Selection and Initial Preparation

Sixty single-rooted mandibular incisors with a single long oval canal were selected for this study. Teeth were obtained from the institutional tooth bank and were extracted for reasons not related to this study. The G*Power 3.1 software (Henrick Heine-Universitat, Dusseldorf, Germany) was used for sample size calculation and demonstrated that 12 specimens per group were necessary to show a 5% difference in results with a power of 80%. The teeth were initially radiographed in buccolingual and mesiodistal projections, and only those with a single long oval canal were selected. A long oval canal was defined as those with a buccolingual-to-mesiodistal ratio >2–4:1 at 5 mm from the apex[20]. In addition, the included teeth showed intact crowns, straight and mature roots, and no evidence of root fracture or resorption. The protocol for this laboratory study was approved by the Institutional Ethics Committee.

Conventional coronal access cavities were prepared and the patency length was established by placing a hand K-type file size 10 (FKG Dentaire, La Chaux-de-Fonds, Switzerland) in the root canal until its tip was visualized at the apical foramen under an operating microscope. The working length (WL) was established 1 mm shorter than the patency length.

Micro-computed Tomographic Scanning

To minimize anatomical differences between specimens, the selected teeth were scanned in a micro-computed tomography (micro-CT) SkyScan 1174v2 device (Bruker-microCT, Kontich, Belgium) using the same parameters as described elsewhere[7]. The teeth were matched in quintets based on anatomic similarities, tooth length, and preoperative root canal volume and surface area, as determined by micro-CT. One specimen from each quintet was then randomly assigned to one of the 5 experimental groups.

Bacterial Contamination of the Root Canals

The root canals were instrumented up to 1 mm beyond the patency length using a hand K-type file size 20 (FKG Dentaire). The canals were then prepared up to the WL with a BioRaCe rotary instrument size 35/04 (FKG Dentaire), operated on a VDW Silver motor (VDW, Munich, Germany) in continuous rotation. Irrigation was performed with 2.5% sodium hypochlorite (NaOCl), delivered by a NaviTip 30-G needle (Ultradent, South Jordan, UT). After preparation, the canals were irrigated with 17% ethylenediaminetetraacetic acid (EDTA), and the teeth were immersed in the same solution for 3 minutes under ultrasonic agitation. Sequential ultrasonic baths were then conducted for 3 minutes each, using distilled water, 2.5% NaOCl, and 10% sodium thiosulfate.

The root canals were washed with distilled water and then completely filled with trypticase soy broth (TSB) (Difco, Detroit, MI). In order to release entrapped air and allow for broth penetration deep into root canal irregularities, the tooth specimens were centrifuged twice within flasks containing the same culture broth at 10,000 rpm for 2 minutes. The specimens immersed in TSB were autoclaved.

The root canals were inoculated with a mixed bacterial culture derived from subgingival biofilm samples collected from an adult volunteer. The samples were cultured in thioglycollate broth with agar for 48 hours. This mixed culture was then added with an aliquot of overnight culture of *Enterococcus faecalis* strain ATCC 29212. For inoculation, the root canals were completely filled with the mixed bacterial culture using NaviTip needles until the broth flowed through the apical foramen. The tooth specimens were eventually immersed in TSB inoculated with the same mixed culture and incubated for 30 days at 37°C under gentle shaking. The culture medium was refreshed weekly.

Sampling Procedures

Afterward, the specimens were removed from the culture medium, wiped with sterile gauze, and their external surfaces were cleaned with 3% hydrogen peroxide and disinfected with 2.5% NaOCl, with care to avoid the apical foramen. After NaOCl inactivation with 10% sodium thiosulphate, Top Dam material (FGM, Joinville, SC, Brazil) was applied to the root apex to simulate the vapor lock effect. The specimens were wrapped in sterile gauze moistened with saline solution and held in a bench vise for handling.

The operative field, including the tooth crown and access cavity walls, were disinfected with 2.5% NaOCl followed by inactivation with sodium thiosulphate. A sterility control sample was collected from the cavity walls by using sterile paper points, which were placed in Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.6). Sterility controls were further processed and analyzed in the same manner as the intracanal samples (see below).

Root canal samples were taken at 3 time points: before (S1) and after one week of intracanal medication (S2), and after a supplementary approach using NaOCl irrigation and agitation with a finishing instrument (S3). Before S1 was taken, the root canal was irrigated with 1 mL sterile saline solution for 1 min using a NaviTip needle. This was carried out to displace and remove unattached bacterial cells. Next, a hand 35/02 instrument (FKG Dentaire) was inserted up to the WL and used in gentle circumferential filing to displace cells adhered to the canal walls and put them in suspension for further sampling. Sterile paper points (sizes 30 and 35) were placed at the WL for 1 min each, and then transferred to 1 mL Tris-EDTA buffer. Sequential sampling with paper points was

continued until no moisture was visible on their surfaces. Samples were stored at −20°C until DNA extraction.

Application of Intracanal Medication

The intracanal medicaments tested were as follows: (1) calcium hydroxide paste in glycerin (CHG); (2) calcium hydroxide paste in camphorated paramonochlorophenol and glycerin (CHPG); (3) calcium hydroxide paste in 2% chlorhexidine gluconate solution (CHCX), and (4) Bio-C Temp (Angelus Indústria de Produtos Odontológicos). The fifth group was a control in which the canals were filled with sterile saline instead of medication.

Calcium hydroxide pastes were prepared by mixing pure calcium hydroxide powder with the liquid in a creamy consistency. For the CHPG paste, the 2 liquids were first mixed in a 1:1 ratio before adding calcium hydroxide powder.

The root canals were filled up to the WL with the test medicaments using a lentulo spiral. Condensers and cotton pellets were then used to pack the medication at the canal opening. If necessary, placement procedures were repeated to ensure a homogeneous filling up to the WL. The access cavity was temporized with Top Dam (FGM), and the specimens were stored in 100% humidity at 37°C.

After 7 days, the operative field was disinfected as outlined earlier, and the temporary coronal seal was removed. A 35/04 BioRace instrument was used in continuous rotary motion with brushing strokes up to the WL to remove the intracanal paste, under irrigation with 3 mL sterile saline. For inactivation of the intracanal medication, 1 mL 10% citric acid was used to irrigate the canal in all groups except for the CHCX group, in which a mixture of 3% Tween 80 and 0.3% soy lectin was used. A hand 35/02 instrument was used in circumferential filing motions before taking the postmedication sample (S2), as described previously.

After S2 sample was taken, the canal was irrigated with 2 mL 2.5% NaOCl and a supplementary approach with the XP-endo Finisher instrument was conducted for 1 min in continuous rotation, driven by the VDW Silver motor (1000 rpm, 1 Ncm). This finishing instrument was used up to 2 mm short of the WL in gentle in-and-out movements.

The canal was finally irrigated with 2 mL 2.5% NaOCl, and 1 mL 10% sodium thiosulphate for 30 seconds. In sequence, a hand 35/02 instrument was used in circumferential filing motions and the S3 bacteriological sample was taken as described above. Samples were stored frozen at −20°C until analysis.

All experimental procedures were performed inside a cabinet (Permution, Curitiba, PR, Brazil) at 37°C maintained by a heater (800-Heater, Plas-Labs, Lansing, MI). Irrigation was always conducted with a syringe and 30G NaviTip needle at a flow rate of 4 mL/min. Irrigants were prewarmed to 37°C and the needle was inserted 2 mm short of the WL.

Molecular Microbiology Analysis

Samples were thawed and subjected to DNA extraction using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Total bacterial levels were quantified in a 16S rRNA gene-based qPCR assay using universal 16S rRNA gene-based primers[21]. The qPCR assay was run using the Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Foster City, CA) on an ABI 7500 Real-time PCR device (Thermo Fisher Scientific), with a 20-μL total reaction volume containing 2 μL DNA extract and 0.5 μM of each primer. The parameters for the qPCR were as follows: initial denaturation at 95°C/10 minutes, and 40 repeats of denaturation at 95°C/1 minute, annealing at 58°C/1 minute, and extension at 72°C/1 minute. The same reaction with no DNA template served as negative control. Reactions were run in triplicate for samples, controls, and standards. A melting curve analysis was performed after amplification to confirm specificity. Data acquisition and analyses were done by using the ABI 7500 software v2.0.4 (Thermo Fisher Scientific). For bacterial counting, a standard curve was prepared with DNA extracted from *E. faecalis* strain ATCC 29212, which was quantified and 10-fold diluted in Tris-EDTA buffer from 10^7 to 10^2 cells.

Statistical Methods

In modeling counts of bacteria after treatment, the negative binomial model with random effects was used to account for data clustering at the individual level. Estimates of incidence rate ratio with 95% confidence intervals were obtained, indicating the changes in bacterial counts over time and also differences in counts relative to the referent group (control). All models were fit using StataSE 18.0 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX). The Fisher's exact test or the chi-square test was used to analyze the incidence of negative results for bacteria in S2 and S3. The statistical significance level was set at $\alpha = 0.05$.

RESULTS

Changes in Bacterial Counts Within Groups (Intragroup Comparisons)

Bacterial counts were significantly reduced from S1 to S2 in all groups, except for the control group (Table 1). As for S1–S3 changes, all groups (control included) showed a significant reduction in bacterial counts.

In the CHPG group, counts significantly decreased by 90.7%, 99.9% and 98.7% between S1 and S2, S1 and S3, and S2 and S3, respectively ($P < .05$).

In the CHCX group, the bacterial counts were significantly reduced by 99.1%, 99.96%, and 96% between S1 and S2, S1 and S3, and S2 and S3, respectively ($P < .05$).

As for the CHG group, the S1–S2 and the S1–S3 bacterial reduction values were 97.6% and 99%, respectively ($P < .05$). The 57.1% decrease from S2 to S3 was not statistically significant ($P > .05$).

Counts in the Bio-C Temp group significantly decreased by 96.5% and 97.3% from S1 to S2 and from S1 to S3, respectively ($P < .05$). However, the S2–S3 reduction was only 22.7%, which was not statistically significant ($P > .05$).

Counts in the control group were not significantly reduced from S1 to S2 ($P > .05$). However, there was a significant decrease of 97% between S1 and S3 and 94.8% between S2 and S3 ($P < .05$).

Changes in Bacterial Counts Between Groups (Intergroup Comparisons)

At S1, there were no significant differences in the bacterial counts between groups (experimental and control). Reduction in S2 counts was significantly higher in all experimental groups when compared with the control group ($P < .05$). There were no significant differences between the experimental groups at S2. In S3, using the control group as the reference, a significant reduction in counts of bacteria was only observed in the CHPG and CHCX groups. S3 counts in the CHCX and CHPG groups were also significantly lower in comparison with Bio-C Temp ($P < .05$). Counts in the CHCX group were significantly lower at S3 than in the CHG group ($P < .05$). Table 2 shows the mean bacterial counting distribution.

Categorical Findings (Presence/Absence)

The number of cases negative for bacteria in S2 was 6/12 (50%) for CHG and CHPG, 3/12 (25%) for CHCX, and 3/11 (27%) for Bio-C Temp. As for S3, all groups had 6 specimens negative for bacteria. There were no significant differences

TABLE 1 - Estimates of Incidence Rate Ratio With 95% Confidence Intervals Showing Changes in Counts of Bacteria Over Time After Treatment With Different Intracanal Medications

Comparisons	IRR (95% CI)	*P* value	IRR (95% CI)	*P* value	IRR (95% CI)	*P* value
Changes within groups (intragroup)	S1–S2		S1–S3		S2–S3	
Control	0.582 (0.054, 6.219)	.654	0.030 (0.003, 0.32)	**.004**	0.052 (0.005, 0.555)	**.014**
CHG	0.024 (0.003, 0.212)	**.001**	0.010 (0.001, 0.091)	**<.001**	0.429 (0.049, 3.726)	.443
CHPG	0.093 (0.011, 0.810)	**.031**	0.001 (0.0001, 0.011)	**<.001**	0.013 (0.002, 0.116)	**<.001**
CHCX	0.009 (0.001, 0.081)	**<.01**	0.0004 (0.0001, 0.003)	**<.001**	0.040 (0.005, 0.346)	**.003**
Bio-C Temp	0.035 (0.004, 0.336)	**.004**	0.027 (0.003, 0.259)	**.002**	0.773 (0.081, 7.400)	.823
Changes between treatments (intergroup)	**S1**		**S2**		**S3**	
ref: Control						
CHG	0.338 (0.034, 3.268)	.349	0.014 (0.001, 0.137)	**<.001**	0.117 (0.012, 1.126)	.063
CHPG	0.364 (0.038, 3.519)	.383	0.058 (0.006, 0.563)	**.014**	0.015 (0.002, 0.144)	**<.001**
CHCX	0.721 (0.075, 6.972)	.778	0.012 (0.001, 0.111)	**<.001**	0.009 (0.001, 0.085)	**<.001**
Bio-C Temp	0.905 (0.089, 9.157)	.932	0.054 (0.005, 0.552)	**.014**	0.811 (0.080, 8.208)	.859
ref: Bio-C Temp						
CHCX	0.798 (0.087, 7.281)	.841	0.211 (0.023, 1.928)	.168	0.011 (0.001, 0.099)	**<.001**
CHPG	0.403 (0.044, 3.675)	.420	1.069 (0.117, 9.758)	.953	0.018 (0.002, 0.168)	**<.001**
CHG	0.374 (0.041, 3.413)	.383	0.259 (0.028, 2.367)	.232	0.144 (0.016, 1.312)	.086
ref: CHG						
CHPG	1.077 (0.124, 9.363)	.946	4.122 (0.474, 35.838)	.199	0.128 (0.015, 1.114)	.063
CHCX	2.133 (0.245,18.545)	.492	0.814 (0.094, 7.082)	.852	0.076 (0.009, 0.658)	**.019**
ref: CHCX						
CHPG	0.505 (0.058, 4.389)	.536	5.060 (0.582, 44.006)	.142	1.693 (0.195, 14.725)	.633

IRR, incidence rate ratio; CI, confidence interval; CHG, calcium hydroxide paste in glycerin; CHPG, camphorated paramonochlorophenol and glycerin.
Bold face, statistically significant ($P < .05$).

between groups when evaluating these categorical data (presence/absence) ($P > .05$).

DISCUSSION

This study used an *ex vivo* model to evaluate the antibacterial effects of a calcium silicate-based intracanal medication and calcium hydroxide associated with different vehicles. The results revealed that all medications tested showed pronounced antibacterial activity and effectively reduced the bacterial counts after 1 week. There were no significant differences between the materials when evaluating the S1–S2 reduction, with all of them being significantly more effective than the control group. Agitation of NaOCl using the XP-endo Finisher further enhanced bacterial reduction in the CHCX and CHPG groups.

Calcium hydroxide has been widely used as an intracanal medication[13]. Its antibacterial effects are mostly related to the ability to alkalinize the surrounding environment. To be effective, the levels of hydroxyl ions released from the paste have to reach toxic concentrations to bacteria. These effects can be limited by the buffer systems occurring in the canal and the surrounding tissues and fluids[13,22]. Because of this, the association of calcium hydroxide with other antimicrobial agents has been proposed[14-19]. However, this study found no significant differences between the different calcium hydroxide pastes, and between them and the bioceramic material. This may be related to the fact that the main effects revealed by the microbiological experimental model used here are mostly focused on the bacterial infection of the root canal walls, where the concentration of hydroxyl ions from the medicaments can reach high levels.

Studies have shown that NaOCl tissue-dissolving effects are increased after previous tissue treatment with calcium hydroxide[23]. In addition, enhanced antibacterial effects have been reported after calcium hydroxide removal and a second preparation step using NaOCl irrigation[24]. In the present study, NaOCl irrigation and agitation with XP-endo Finisher following calcium hydroxide removal led to significantly improved bacterial reduction in the groups using biologically active vehicles (CHPG and CHCX). In addition to the pronounced antibacterial effects of NaOCl, the possibility exists that these pastes may have effects on the biofilm structure in such a way that further exposure to NaOCl could improve elimination. Further studies should elaborate on the effects of calcium hydroxide pastes in different vehicles on bacterial biofilms.

Bio-C Temp demonstrated antibacterial efficacy comparable to calcium hydroxide pastes, a result that contrasts with previous studies suggesting an inferior antimicrobial efficacy for Bio-C Temp[25,26]. A study demonstrated that this bioceramic dressing showed alkaline pH and dose and time-dependent cytotoxic effects[27], which might explain the observed results. The differences from previous studies may be related to the different methodologies used and the targeted bacteria. Here, we tried to simulate better the clinical conditions by using extracted teeth and a mixed bacterial culture from the subgingival crevice.

The main strengths of this study are related to the fact that natural human teeth were used, better replicating the clinical

TABLE 2 - Mean Distribution of Bacterial Counts in Each Treatment Group at Each Time Point

	Study time point		
Treatment	**S1**	**S2**	**S3**
Control	1.66×10^7	9.67×10^6	5.02×10^5
CHG	5.62×10^6	1.37×10^5	5.86×10^4
CHPG	6.05×10^6	5.63×10^5	7.50×10^3
CHCX	1.20×10^7	1.11×10^5	4.43×10^3
Bio-C Temp	1.50×10^7	5.27×10^5	4.07×10^5

condition for bacterial colonization and medicament interactions. The specimen anatomy was evaluated and standardized based on micro-CT scans, in an attempt to diminish the risks of biases posed by anatomic issues. A mixed bacterial culture was used because root canal infection is usually composed of multiple species, and *E. faecalis* was added because this species is frequently detected in teeth with post-treatment apical periodontitis[28], can colonize the dentinal walls and tubules[29], and may be resistant to some root canal procedures[30].

One of the limitations of this study is its *ex vivo* nature. While this allowed for specimen standardization, it did not fully replicate clinical variables that could influence outcomes. Root canal preparation was completed before contamination to standardize conditions for bacterial colonization, but this meant that the tested medications faced a higher bacterial load than would typically be present immediately after root canal preparation in the real clinical setting.

In conclusion, this study demonstrated that all medications tested promoted a substantial reduction in bacterial counts, with no significant differences between them. A final NaOCl irrigation and agitation using XP-endo Finisher significantly enhanced disinfection in the CHCX and CHPG groups.

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The authors deny any conflicts of interest related to this study.

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