# Antimicrobial Activity and Physicochemical Properties of Calcium Hydroxide Pastes Used as Intracanal Medication

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## Abstract

**Introduction:** The aim of the present study was to evaluate the pH, calcium release, solubility, and antimicrobial action against biofilms of calcium hydroxide + saline solution, Calen (SS White Artigos Dentários Ltd, Rio de Janeiro, Brazil) (CH/P), Calen camphorated paramonochlorophenol (CMCP) (CH/CMPC), and calcium hydroxide + chlorhexidine (CH/CHX) pastes. **Methods:** The pH of the pastes was determined with a calibrated pH meter placed in direct contact with each paste. The root canals of acrylic teeth ($N$ = 10) were filled with the previously mentioned intracanal dressings and immersed in ultrapure water to measure hydroxyl (pH meter) and calcium ion release (atomic absorption spectrophotometer) at time intervals of 3, 7, 15, and 30 days. To assess solubility, the root canals of acrylic teeth ($N$ = 10) were filled with the previously mentioned pastes and scanned by micro–computed tomographic imaging before (initial) and after 7, 15, and 30 days of immersion in ultrapure water. The solubility of each specimen was the difference between the initial and final volume scanning. For antimicrobial analysis, monospecies and dual-species biofilms were *in vitro* induced on dentin blocks ($N$ = 20). Afterward, they were treated with the pastes for 7 days. Live/dead dye and a confocal microscope were used to measure the percentage of living cells. Data were statistically compared ($P$ < .05). **Results:** The highest $OH^-$ ion release values were found in 3 and 30 days. $Ca^{2+}$ releases were greater in CH/CMCP. CH/P and CH/CMCP showed a higher percentage of volume loss values. CH/CHX presented the greatest antimicrobial action. **Conclusions:** CH/P and CH/CMPC showed higher solubility values in the period analyzed. Seven days of contact may be insufficient for calcium hydroxide + saline solution, CH/P, and CH/CMCP pastes to kill bacterial cells in the biofilms studied. Chlorhexidine added to CH favored greater effectiveness against the previously mentioned bacterial biofilms. *(J Endod 2016;■:1–7)*

## Key Words

Antimicrobial action, biofilms, calcium hydroxide paste, intracanal medication, physicochemical properties

**Significance**

The essential basis for the choice of an intracanal dressing is the knowledge of its physicochemical characteristics and antimicrobial action. Thus, the aim of this study was to analyze the influence of vehicles on $OH^-/Ca^{2+}$ ion release, solubility, and antimicrobial action of calcium hydroxide pastes against biofilms.

Root canal cleaning and shaping effectively reduce microbiota in infected teeth but not sufficiently to obtain complete antisepsis; therefore, intracanal dressing is indicated. Calcium hydroxide (CH) powder in a vehicle has been widely used for this purpose. As a slow-working antiseptic, the definite time required for its antimicrobial and biologic actions, by ionic dissociation of $Ca^{2+}$ and $OH^-$ ions, is unknown (1).

Inert vehicles associated with CH may be ineffective to kill *Enterococcus faecalis,* a bacterium commonly isolated in persistent root canal infections, presenting resistance to alkaline pH (2, 3). Furthermore, this microorganism's capacity for adhering, colonizing, and forming biofilms favors associations between species, increasing their resistance to antimicrobial substances (4).

To enhance the antiseptic action of CH paste, an association with antimicrobial vehicles has been suggested. Camphorated paramonochlorophenol (CMCP), a widely used substance, can increase the bactericidal spectrum of CH and promote deeper paste penetration into the dentin (5). The benefits of adding chlorhexidine (CHX), another substance that has been associated with CH paste (6) and has shown activity against gram-positive and gram-negative bacteria, remain unclear and controversial, particularly against microbial biofilm.

The vehicle mixed with the CH determines the velocity of ionic dissociation (7), solubility, and diffusibility, and although some vehicles provide faster dissociation, and, consequently, faster alkalinity of dentin and apical and periapical tissues, they may make the paste more caustic and aggressive in direct contact with tissues (8).

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However, the controlled release of $Ca^{2+}$ and $OH^-$ ions to the apical and periapical tissue contributes to their repair.

Based on the foregoing, the objectives of this study were to evaluate the pH and calcium release of different intracanal dressings, the percentage of volumetric loss, and the antimicrobial effect on monospecies (*Enterococcus faecalis*) and dual-species (*E. faecalis* + *Pseudomonas aeruginosa*) biofilms. The null hypothesis tested was that the pastes would present similarity in pH, calcium release, solubility, and antimicrobial action.

## Methods

### CH Pastes

Four CH pastes were evaluated:

1. G1 (CH/S): CH (Merck & Co, Kenilworth, NJ)/saline (1 gr/0.8 mL)
2. G2 (CH/P): CH/polyethylene glycol (Calen; SS White Artigos Dentários Ltd, Rio de Janeiro, Brazil)
3. G3 (CH/CMCP): CH/polyethylene glycol/CMCP (Calen PMCC, SS White Artigos Dentários Ltd)
4. G4 (CH/CHX): CH ((Merck & Co)/2% CHX gluconate/propylene glycol (1 g/0.5 mL/0.3 mL)

### Experiments 1, 2, and 3: pH of the Pastes and Hydroxyl and Calcium Ion Release

The pH of the pastes was determined with a calibrated pH meter standardized with buffer solutions 4, 7, and 12. For test 1, the pastes (4 groups) were manipulated and immersed into 10 mL distilled water and agitated for 5 minutes. Next, the pH of the solutions was measured with a pH meter (model 371; Micronal, São Paulo, SP, Brazil). For tests 2 and 3, 40 artificial acrylic resin maxillary central incisors ($N = 10$) with an artificial foramen standardized to a diameter of 400 $\mu$m were filled with the pastes of the respective groups analyzed. Each tooth, individually immersed in a plastic bottle containing 10 mL ultrapure water, was moved to a new plastic bottle with an equal volume of new ultrapure water after time intervals of 3, 7, 15, and 30 days. The pH and calcium release of the solutions were analyzed with a pH meter (model 371) and an atomic absorption spectrophotometer (AA6800; Schimadzu, Tokyo, Japan) equipped with a calcium ion–specific hollow cathode lamp, respectively. This methodology was based on Duarte et al's method (9).

### Experiment 4: Micro–computed Tomographic Volumetric Solubility

For solubility analysis, the root canals of 40 acrylic teeth (with the same standardization as in experiments 2 and 3) were filled with the experimental pastes ($N = 10$), and the coronal access was sealed with Bioplic (Biodinâmica, Londrina, Brazil). Immediately afterward, the samples were scanned with a desktop x-ray micro-focus computed tomographic scanner (SkyScan 1174v2; SkyScan, Kontich, Belgium). The image capture parameters were as follows: voxel size of 19.70 $\mu$m, 0.5° rotation steps, and a 360° rotation. Each scan consisted of 373 TIFF images with 1024 × 1304 pixels. Subsequently, the samples were individually immersed in plastic bottles containing 10 mL deionized water and stored at 37°C. At time intervals of 7, 15, and 30 days, the acrylic teeth were removed from their bottles, and new scanning was performed using the same parameters used in the first stage. The scanned images obtained were reconstructed, and the volume ($mm^3$) of the pastes was measured with CTan software (CTan v1.11.10.0, SkyScan). The solubility values of each specimen was as follows: initial − final volume (scanning equal to the total volume lost during immersion). The solubility percentage was calculated by dividing the volume lost by the total volume.

### Experiment 4: Antimicrobial Test and Microscopic Analysis

**Biofilm Growth.** Microbiological procedures and manipulation of the pastes were conducted under aseptic conditions in a laminar flow chamber (VecoFlow Ltda, Campinas, SP, Brazil). For *E. faecalis* biofilm, 15 $\mu$L standard strain (American Type Culture Collection [ATCC] 4083) were put into 3 mL sterile brain-heart infusion (BHI) (Oxoid, Basingstoke, UK) at 37°C in air for growth overnight. For dual-species biofilm, the same procedure described was used with *E. faecalis* (ATCC 4083) and *P. aeruginosa* (ATCC 10145), each one separately and at different times to ensure that no contamination occurred. After growth overnight, bacterial density was adjusted at $10^9$ cells/mL for *E. faecalis* (ATCC 4083) and $10^7$ cells/mL for *P. aeruginosa* with a spectrophotometer (UV-VISIBLI, Shimadzu, Japan) at an optical density of 1 at 600 nm according to the 0.5 MacFarland standard.

**Dentin Surface Infection.** Dentin blocks, obtained from bovine central incisors with fully developed roots using trephine drills 4.0 mm in diameter under abundant irrigation, were subsequently sterilized in an autoclave. After density adjustment, the dentin surfaces were infected. For *E. faecalis* biofilm, 1 dentin block + 100 $\mu$L *E. faecalis* + 900 $\mu$L BHI were inserted into each well of a 24-well multiwell plate. For dual-species biofilms, 1 dentin block + 100 $\mu$L *E. faecalis* + 100 $\mu$L *P. aeruginosa* + 1500 $\mu$L BHI were put into each well according to van der Waal et al's methodology (10). For growth of both types of biofilms, all plates were incubated aerobically at 37°C for 21 days (monospecies biofilm) and 4 days (dual-species biofilm). The BHI was refreshed every 2 days.

**Antimicrobial Test for *E. faecalis* Biofilm and *E. faecalis* + *P. Aeruginosa* Biofilm.** After the incubation period, infected samples were washed with 1 mL distilled water to remove loosely adherent planktonic bacteria. Afterward, they were randomly divided into 6 groups ($N = 20$) according to the experimental pastes and a control group without treatment. For the contact test, dentin samples were immersed in the experimental intracanal dressings and incubated at 37°C for 7 days.

**Microbiological Analysis.** Biofilm viability was analyzed using the SYTO 9/propidium iodide technique (Live/Dead BacLight Viability Kit; Molecular Probes, Eugene, OR). After the established time in contact with the pastes, the blocks were washed with phosphate-buffered saline and stained in a dark environment with 15 $\mu$L of the dyes for 15 minutes. Then, they were washed again and directly observed by inverted confocal laser scanning microscopy (Leica TCS-SPE; Leica Biosystems CMS, Mannheim, Germany). Four confocal "stacks" of random areas were obtained for each sample with a 40× oil lens. In total, there were 5 samples per group, thus 20 stacks for each medication. For quantification, bioImage_L software (www.bioImageL.com) was used to calculate the total biovolume and the percentage of red (dead cells) found after the antimicrobial treatment.

### Statistical Analysis

The Shapiro-Wilk test was used to verify the normality of data from all analyses, and absence of normality was observed. Therefore, statistical comparisons between the groups were made by the Kruskal-Wallis and Dunn tests. The significance level was established at 5%.

## Results

Table 1 shows the $OH^-$ and $Ca^{2+}$ ion release results (tests 2 and 3). The highest $OH^-$ ion release was observed at 3 and 30 days. CH/CMCP presented the highest $Ca^{2+}$ release. At 7 and 30 days, no