



ORAL SURGERY

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**ENDODONTICS***Editor: Larz S. W. Spångberg***Antimicrobial activity of extracts of calcium hydroxide points**

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The purpose of this study was to evaluate in vitro the antibacterial effect of calcium hydroxide points by using a broth dilution test. Extracts of calcium hydroxide points and a calcium hydroxide paste (Calasept) were tested by using 2 facultative anaerobic bacteria, *Streptococcus mutans* (ATCC 27352) and *Enterococcus faecalis* (ATCC 29212). Saline solution was used as a control. Each tested agent was kept in contact with the bacterial species used for the experiment for 5 minutes, 1 hour, 1 day, 2 days, and 5 days. Results showed that Calasept paste was effective in killing the tested bacteria, whereas calcium hydroxide points and saline showed bacterial survival in all experimental periods. It was concluded that calcium hydroxide points extract is not an effective antimicrobial agent against the tested bacteria.

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Complete chemomechanical preparation of the infected root canal system is essential to reduce the population of microorganisms. However, some microorganisms may be difficult to eliminate with mechanical instrumentation because of growth in dentinal tubules, lateral canals, and apical ramifications.<sup>1-3</sup> Therefore, there is a need to use an intracanal medicament to help eliminate or reduce the number of microorganisms.

Various medicaments such as phenolic compounds and calcium hydroxide have been used as root canal disinfectants. Calcium hydroxide has been recommended as the antimicrobial agent of choice in endodontic therapy<sup>4</sup> because of its superiority to other antimicrobial agents. Ideally, calcium hydroxide should be spread thickly inside the entire canal space so that it is close to the bacteria. The pretreatment of infected root canals with calcium hydroxide improves the prognosis.<sup>5</sup> Calcium hydroxide points were recently introduced on the market as temporary filling points for medication. However, no reports have yet

been published evaluating the antibacterial activity of calcium hydroxide points. Therefore, the purpose of this study was to evaluate the antibacterial effect of calcium hydroxide points by using a tube dilution test.

**MATERIAL AND METHODS**

The antibacterial effects of the calcium hydroxide extract solutions were evaluated on 2 facultative anaerobic bacteria, *Enterococcus faecalis* (ATCC 29212) and *Streptococcus mutans* (ATCC 27352). Calcium hydroxide points (batch #149808; Roeko, Langenau, Germany) and calcium hydroxide paste (Calasept, batch #10001; JS Dental Manufacturing, Inc, Ridgefield, Conn) were used in this study. In addition, sterile saline solution (batch #197040; Pharmaceutical sol. Industries Ltd, Jeddah, Saudi Arabia) was used as a control.

The calcium hydroxide points were cut into small pieces and submerged in saline for 1 hour. Thirty points were used for each 10 mL of saline, and the mixture was agitated well every 15 minutes for 1 hour. The pieces of the calcium hydroxide points were discarded, and the remaining solution was used for the experiment. One carpule of Calasept (1.8 mL) was mixed in 10 mL of saline. The mixture was agitated well every 15 minutes for 1 hour. The mix was allowed to set for 15 minutes. The supernatant was used for the experiment. The pH of the solutions was measured before the start of the experiment.

The bacterial strains were grown in prereduced anaerobically brain-heart infusion broth supplemented

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**Table I.** Evaluation of the effect of the direct contact of tested materials on cultured *Enterococcus faecalis*

Culture period	Contact time														
	Control (saline solution)					Calasept paste					Ca(OH) <sub>2</sub> points				
	5 min	1 h	1 d	2 d	5 d	5 min	1 h	1 d	2 d	5 d	5 min	1 h	1 d	2 d	5 d
1 d	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
2 d	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
3 d	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
4 d	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
5 d	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+

N = 5 per test.

+, Presence of growth; -, absence of growth.

**Table II.** Evaluation of the effect of the direct contact of tested materials on cultured *Streptococcus mutans*

Culture period	Contact time														
	Control (saline solution)					Calasept paste					Ca(OH) <sub>2</sub> points				
	5 min	1 h	1 d	2 d	5 d	5 min	1 h	1 d	2 d	5 d	5 min	1 h	1 d	2 d	5 d
1 d	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
2 d	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
3 d	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
4 d	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
5 d	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+

N = 5 per test.

+, Presence of growth; -, absence of growth.

with hemin and menadione. Inoculum was prepared in this culture medium by adjusting to an 0.5 McFarland BaSO<sub>4</sub> turbidity standard.

For the test, 1 mL of the prepared extract solution of calcium hydroxide point or paste was placed at the bottom of the wells of 24-well cell culture plates. Five wells were used per test. Wells containing 1 mL of 0.9% sterile saline served as the controls. Subsequently, 1 mL of culture of the bacteria to be tested was placed into the wells containing calcium hydroxide extract or saline. The cell culture plates were incubated at 37°C for 5 minutes, 1 hour, 1 day, 2 days, and 5 days.

At the end of the experimental time, 0.1-mL aliquots were taken from each well and transferred to tubes containing 5 mL of fresh brain-heart infusion broth supplemented with hemin and menadione. All the tubes were vortexed and then incubated at 37°C and observed for 5 days.

The bacteria were observed daily for growth, which was indicated by the presence of turbidity in the tubes. To control for contamination, all cultures with growth were cultured on blood agar plates and the morphology was evaluated.

## RESULTS

The pH of the calcium hydroxide points extract was 12.5 and 13.0 for Calasept. The saline solution had a pH of 6.8.

From the data shown in Tables I and II, it can be observed that the supernatant of the Calasept preparation was effective in killing the bacteria tested, whereas the extract from calcium hydroxide points showed bacterial growth at all experimental periods. No sample had contamination.

## DISCUSSION

The dilution tube susceptibility test, which is an effective method to evaluate the antibacterial properties of antimicrobial agents, was used.<sup>6</sup> This method allows direct contact in the solution between bacterial cells and the agent to be tested. Such a method proves critical when evaluating the antimicrobial activity of calcium hydroxide, which has a low solubility and diffusibility. In a study in vitro, Byström et al<sup>4</sup> reported that most bacteria found in infected root canals die within 1 to 6 minutes when placed in direct contact with calcium hydroxide.

*E faecalis* and *S mutans* were chosen as test organisms in this study. *E faecalis* is among the few facultative anaerobic microorganisms associated with persistent apical periodontitis<sup>7,8</sup> and is the most resistant species to eliminate from root canals.<sup>9</sup>

The results of this experiment showed that the extract from Calasept was very effective in killing the tested microorganisms at all observation periods. These findings are in agreement with those of other

studies.<sup>4,10,11</sup> Calcium hydroxide kills bacteria because of the alkaline effects of hydroxyl ions, which are believed to be responsible for the antibacterial activity of calcium hydroxide.<sup>12</sup> This will increase the membrane permeability of the bacteria, as well as protein denaturation and damage to the DNA.<sup>13</sup> In a clinical study, Byström et al<sup>4</sup> reported that living bacteria could only be recovered from 1 of 35 infected root canals treated with calcium hydroxide for 1 month.

The extracts from calcium hydroxide points had no measurable antibacterial effect, similar to the saline solution. The tested microorganisms survive in all of the experimental periods in the extracts of the calcium hydroxide points, even though the pH of the extract was almost as high as the pH of the Calasept extract. The reason for this lack of antimicrobial activity must be found in the inactivating effect of the bacterial cells on the extract from the calcium hydroxide points. Thus, the hydroxide ions in the calcium hydroxide points solution became insufficient despite the initially measured pH.

Our findings are in agreement with those of Byström et al,<sup>4</sup> who showed that the short application of calcium hydroxide dressing is very effective. Sjögren et al<sup>14</sup> reported that a period of 7 days might be sufficient to ensure eradication of the bacteria.

Calt et al<sup>15</sup> evaluated the diffusion properties of Ca<sup>2+</sup> and OH<sup>-</sup> ions through root dentin in vitro. They found that the root canals filled with calcium hydroxide points did not release appreciable amounts of Ca<sup>2+</sup> into the media. In addition, they observed that the calcium hydroxide points scored the lowest pH value among calcium hydroxide dressing materials. They concluded that calcium hydroxide points are not useful therapeutic dressing materials for the treatment of root resorption. This study recapitulates that the calcium hydroxide point is a poor vehicle for the delivery of calcium hydroxide as an effective intracanal antimicrobial agent.

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