

**Antimicrobial Activity of Various Calcium
Hydroxide Forms as Intracanal
Medicament at Various Dentine Depths
(In vitro study)**

Thesis

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تأثير النشاط المضاد البكتيري لأنواع مختلفة من الكالسيوم هيدروكسيد المستخدمة كعقار طبي مؤقت داخل القنوات الجذرية وعلى مستويات مختلفة من طبقة العاج

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The success of the root canal treatment is dependent on the removal of bacteria from the root canal space and prevents re-infection.

The irrigation and mechanical cleaning of root canal are the most important in reducing the amount of bacteria during the endodontic treatment, however it has proved difficult to obtain complete disinfection in all cases. Bacteria remaining in obturated root canal may proliferate and invade ramifications, apical deltas, isthmuses and dentinal tubules. In these locations bacteria remain unaffected by chemomechanical preparation and may result in persistent endodontic infections.

Therefore, the use of intracanal medicaments is essential to prevent or at least delay the re-infection between the sessions and so, any medication chosen for intracanal application must be evaluated as it's antimicrobial activity by means of in-vitro successibility tests.

In recent years, calcium hydroxide has emerged a popular choice of intracanal medicaments. The antibacterial effectiveness of calcium hydroxide is due partly to its high pH(~12) which prevents survival of many oral bacteria, calcium hydroxide alters the biologic properties of bacterial lipopolysaccharides in the cell

walls of Gram negative species and inactivates membrane transportation mechanisms, resulting in cell toxicity.⁽²³⁾

The primary advantage of calcium hydroxide is its ability to kill micro-organisms in the absence of direct contact by absorbing the CO₂ required for bacterial growth and by the release of hydroxyl ions, which diffuse into dentinal tubules.

Despite the success of calcium hydroxide as an intracanal medicament, several bacterial species including *Enterococcus Faecalis* are reported to be resistant to its effects, so substance with various antibacterial and chemical properties have been used to evaluate their effects against *E.faecalis*. Recently a formula of calcium hydroxide was introduced (metapex 30% calcium hydroxide , 40.4% iodoform , 22.4% silicone oil) as intracanal medicament as it is found to be effective against *E.faecalis*.

From previous studies the iodoform has been indicated as an antiseptic all over the years due to iodine release in nascent state when in contact with secretions or endodontic infections. For different reasons iodoform has been used mixed to calcium hydroxide to increase its antimicrobial activity.

Therefore designing a study to investigate the antibacterial activity of various forms of Ca(OH)₂ as intracanal medicament was thought to be of value.

I- Use of Intracanal Medicaments :-

Haapasalo et al ⁽¹⁾ showed that dentine powder had an inhibitory effect on all medicaments i) saturated Ca(OH)_2 in water. ii) 1% sodium hypochlorite. iii), 0.5% and 0.05% chlorhexidine acetate. iv) iodine potassium iodide in 2/4% tested, which was dependent on the concentration of the medicaments as well as on the length of the time the medicament was preincubated with dentine powder before adding the *E.faecalis*. The effect of 0.05% chlorhexidine and 1% sodium hypochlorite on *E. faecalis* was reduced but not totally eliminated by the presence of dentine.

Weiger et al ⁽²⁾ explored the influence of calcium hydroxide as an interappointment dressing on the healing of periapical lesions associated with pulpless teeth that had not been endodontically treated previously by comparing the prognosis after two-visit root canal treatment with that following a one-visit treatment. Seventy-three patients were divided into 2 groups ,one group had been received one visit root canal treatment,& the other group had received 2 visits with Ca (OH)_2 in between the visits. The criteria for success were the absence of signs and symptoms indicating an acute phase of periapical periodontitis and radiographically a periodontal ligament space of normal width. They concluded that one-visit root canal treatment created

favourable environmental conditions for periapical repair similar to the two-visit therapy when calcium hydroxide was used as antimicrobial dressing. One-visit root canal treatment is an acceptable alternative to two-visit treatment for pulpless teeth associated with an endodontically induced lesions.

Pérez et al ⁽³⁾ measured variations in dentinal pH following the placement of various forms of calcium hydroxide in either the root canal or the pulp chamber. Three cavities were drilled through the root dentine to within 1 mm of the canal wall at the cervical, middle and apical thirds, then divided into five groups; group 1: pure aqueous calcium hydroxide paste , group 2: the same aqueous calcium hydroxide paste was placed in the pulp chamber; group 3: Hycal, a new form of calcium hydroxide paste, was placed in the pulp chamber; group 4: calcium hydroxide gutta-percha points were placed in the root canal; group 5: control group, wet canal (distilled water) without medication. The Ph was measured in the dentinal cavities at 8 h and at 1, 2,3, 7, 14, and 21 days using a calibrated microelectrode. The results showed that At 7 days, the pH had increased in the Hycal group without being significantly different from the aqueous calcium hydroxide paste placed either in the root canal or in the pulp chamber. At 14 days, Hycal had the highest pH values. They concluded that the aqueous calcium hydroxide paste placed in the root canal or Hycal had similar values at days 7 and 21. an aqueous calcium hydroxide

paste placed in the pulp chamber increased dentinal pH more than the other techniques.

Estrela et al ⁽⁴⁾ determined the influence of vehicles on the antimicrobial efficiency of calcium hydroxide. A total of 588 size 50 sterile absorbent paper points, were immersed in various microbial suspensions for 3 min. then were placed on Petri dishes and covered with intracanal dressings containing calcium hydroxide: Ca(OH)_2 + saline; Ca(OH)_2 + camphorated-paramonochlorophenol; Ca(OH)_2 + 1% chlorhexidine solution; Ca(OH)_2 + 3% sodium lauryl sulphate; Ca(OH)_2 + Otosporin. After 1 min, 48 and 72 h and 7 days. Some of absorbent paper cones were removed from contact with the intracanal dressings and individually transported and immersed in 5 mL of Letheen Broth, & Microbial growth was evaluated by turbidity of the culture medium. The results showed that an antimicrobial effect occurred after 48 h. They concluded that the various vehicles associated with calcium hydroxide pastes did not influence the time required for microbial inactivation.

Han et al ⁽⁵⁾ investigated the antibacterial activity of Ca(OH)_2 containing pastes with *Enterococcus Faecalis* in vitro. Sixty-eight standardized human root specimens were infected with *Enterococcus faecalis* for 3 weeks after removal of the smear layer. After 3 weeks of infection, the smear layer was reformed.

Aqueous $\text{Ca}(\text{OH})_2$ paste and silicone oil based $\text{Ca}(\text{OH})_2$ paste were used as the test medications. The results showed that after removal of medications dentin chips were collected and incubated after 7 days. All calcium hydroxide pastes were effective in the elimination of bacteria in the dentinal tubules, except in the smeared group with silicone oil-based calcium hydroxide. They concluded that the presence of a smear layer delayed, but did not eliminate, the effect of the medicaments. The hydroxyl ion of calcium hydroxide diffused more rapidly through radicular dentin when the smear layer was removed from the canal walls.

Estrela et al ⁽⁶⁾ analyzed two methods for determining the antimicrobial effectiveness of (i) calcium hydroxide plus saline, (ii) calcium hydroxide plus polyethylene glycol, and (iii) calcium hydroxide plus camphorated paramonochlorophenol. Four microorganisms (*Staphylococcus aureus*, *Enterococcus Faecalis*, *Pseudomonas aeruginos*, and *Bacillus subtili*, one yeast (*Candida albican*) and one mixture of these organisms were used. The strains were inoculated in Brain Heart Infusion (BHI) and incubated at 37°C for 24h. two methods, the direct exposure test and the agar diffusion test were used to evaluate antimicrobial effects. The inhibition zone around each well was recorded in millimeters. The results showed that intracanal dressings induced inhibition zones. Data obtained showed that both the DET and agar diffusion test are useful in protocols.