

Antimicrobial Efficacy of Four Calcium Hydroxide Formulations and Chlorhexidine Gel using Agar Diffusion Model

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Citation

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Abstract

Aim: We sought to assess, in vitro, the antimicrobial efficacy of four Calcium hydroxide formulations and chlorhexidine (CHX) gel against *E. faecalis*. **Materials and Methods:** The effect of CHX gel and Calcium hydroxide in combination with various vehicles was evaluated by using the agar diffusion test, to measure the zone of inhibition. Plates of BHO agar were inoculated with *E. faecalis*. Each plate had six wells saturated with the medicaments and the center well without the medicament served as the control. The samples were incubated at 37° C for 24 hrs in an incubator. The largest diameter of the zones of microbial inhibition was measured and recorded. Statistical analysis was performed using One-way ANOVA followed by multiple Tukey HSD method. **Results:** In the agar diffusion test, 1% chlorhexidine Gluconate gel showed better antibacterial efficacy ($p < .05$) against *E. faecalis*. Vitapex paste with 30% $\text{Ca}(\text{OH})_2$ showed least inhibition of *E. faecalis*. **Conclusion:** CHX is effective against *E. faecalis* in vitro. Further in vivo studies are needed to confirm the value of CHX in clinical treatment.

INTRODUCTION

The main aim of endodontic treatment is to eliminate microorganisms and their byproducts from the root canal system as well as to prevent re-infection. Though cleaning and shaping plays an important role, it is not capable of thoroughly eliminating microorganisms, particularly anaerobic bacteria¹. Occasionally, treatment-resistant species, especially facultative anaerobe *Enterococcus faecalis*, become dominant and difficult to eradicate from the root canal.

Investigators have noted that bacteria in instrumented, unfilled canals can multiply and reach their pretreatment numbers in 2 to 4 days. Hence, it is generally believed that residual microorganisms can further be reduced by dressing the canal with a medicament between successive treatment sessions^{2,3}. Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is commonly used for this purpose; however, specific microbes, such as *E. faecalis*^{3,4} and *Candida albicans*⁵, are resistant to Calcium hydroxide. Furthermore, recent researchers have questioned the efficacy of Calcium hydroxide in reducing microbial numbers even after prolonged contact with the root canal⁶.

Chlorhexidine (CHX) has a wide-spectrum antimicrobial activity against gram-positive and gram-negative microorganisms, bacterial spores, lipophilic viruses and yeasts⁷. As a root canal irrigant and intracanal medicament, CHX has an antibacterial efficacy comparable to that of sodium hypochlorite (NaOCl)^{8,9}. In addition, it is also effective against strains resistant to Calcium hydroxide¹⁰.

The aim of the present in vitro study was to assess the antimicrobial efficacy of different concentrations of $\text{Ca}(\text{OH})_2$ in combination with various vehicles and chlorhexidine gel against *E. faecalis*.

MATERIALS AND METHODS

AGAR DIFFUSION TEST

E. faecalis (ATCC 292121) was grown overnight in brain-heart infusion (BHI) broth, adjusted to a 0.5 turbidity reading on the McFarland scale (1.5×10^8 bacteria/mL), and inoculated in BHI agar plates. Inoculation was performed by using sterile pipettes brushed across the media. Six round wells, 5 mm deep and 6 mm in diameter, were punched in the cultivated agar plates with a sterile copper tube. Five wells were made on periphery while one at the center

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without medicament served as the negative control. The medicaments, their concentrations, and the delivery vehicles are described below:

Group I - 99.9% Ca(OH)₂ powder(Calcium hydroxide extra pure, DPI, India) mixed with saline – 1gram/ml

Group II - 99.9% Ca(OH)₂ powder(Calcium hydroxide extra pure, DPI, India) with 1% chlorhexidine solution – 1gram/ml

Group III (Vitapex, Neodental International Inc, Japan)) - 30% Ca(OH)₂ paste with 22.4% silicon base

Group IV (Apexcal, Ivoclar Vivadent, Liechtenstein) - 25% Ca(OH)₂ in a mixture of water glycerin, polyethylene glycol and other supplementary agents.

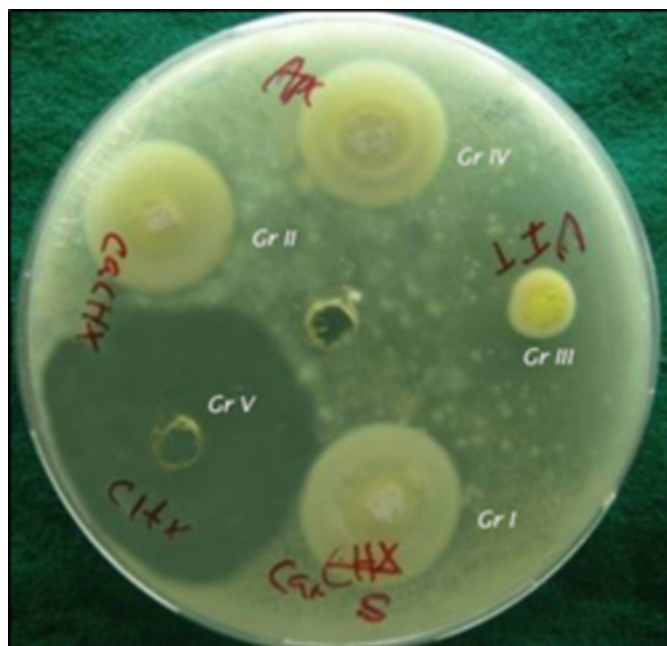
Group V (Hexigel, ICPA, India) - 1% Chlorhexidine Gel

A sample from each group was placed in the outer five wells. A total of six such inoculated agar plates with medicaments were prepared.

The agar plates were incubated at 37 ° C for 24 hrs in an incubator. The diameters of bacterial inhibition zones around each well were measured and recorded (Fig. 1).

Figure 1

Fig.1 Agar plate showing the zones of inhibition



The 5mm well widths were subtracted from the measurements, which were then divided by a factor of 2 to obtain the average zones of inhibition on one side of the

wells.

RESULTS

Statistical analysis using One- way ANOVA followed by using multiple Tukey HSD method revealed significant difference ($P < 0.05$) between the groups (Table 1).

Figure 2

Table -1 Mean average zones of inhibition

Groups	Number of specimens	Mean	Standard deviation
Calcium hydroxide and saline(Gr I)	6	4.9250	.34315
Calcium hydroxide and CHX(Gr II)	6	5.3750	.49371
Vitapex(Gr III)	6	2.0853	.52002
Apexcal(Gr IV)	6	4.6500	.37417
1% CHX gel(Gr V)	6	9.9250	.41683
Total	30	5.3867	2.62159

1% chlorhexidine gel showed significant inhibition of bacterial growth compared with other medicaments. Vitapex showed least inhibition of bacterial growth. No significant difference was found between Ca(OH)₂ combined with saline, Calcium hydroxide combined with 1% chlorhexidine solution and Apexcal.

DISCUSSION

E. faecalis was selected due to its high prevalence in therapy resistant cases and root treatment failures¹¹. In the present study, 1% chlorhexidine showed higher mean zone of inhibition compared to other medicaments. This is in accordance with the study done by Heling et al.¹² which concluded that chlorhexidine was more effective than Ca(OH)₂ in eliminating *E. faecalis* infection inside dental tubules.

Apexcal with 25% Ca(OH)₂ showed no significant difference between 99.9% Calcium hydroxide mixed with saline and 1% chlorhexidine solution. Safavi and Nakayama (2000) stated that thick preparations of Ca(OH)₂ should have the same ionic concentration as thin preparations of Calcium hydroxide. There may be other factors for antibacterial effect as vehicle for mixing and consistency of the mix. Calcium hydroxide mixed with 1% chlorhexidine has the same antimicrobial effect as when mixed with saline.

Chlorhexidine has a positive charge and Calcium hydroxide has a negative charge. Therefore, the pH effect of Calcium

hydroxide is possibly decreased when mixed with chlorhexidine. The same interaction could have decreased the effectiveness of chlorhexidine in this formulation.

Our study showed that CHX had good efficacy against *E. faecalis*. The agar diffusion test suggested that the efficacy is concentration-dependent. When considered jointly with the results of previous studies evaluating *E. faecalis* or other microorganisms^{13,14}, these results confirmed the excellent potential of CHX when applied as an intracanal medicament. It would appear that the clinically desirable goal of root canal disinfection can be achieved by using CHX as an intracanal medicament. CHX has several properties that suggest it to be a suitable alternative to Calcium hydroxide as an intracanal medicament. Compared with, $\text{Ca}(\text{OH})_2$ it has a broader antimicrobial spectrum¹⁵. Even at the higher concentrations it has very low toxicity¹⁶. Because of its cationic properties, CHX is adsorbed onto hydroxyapatite in the dentin^{17,18} and may subsequently be released. This process of CHX uptake and release leads to substantive antimicrobial activity that may protect the root dentin against microbial colonization beyond the actual medication period. Applied in solution or as a thin gel, CHX may reach irregularities in the root canal system better than $\text{Ca}(\text{OH})_2$ paste. Also in contrast to Calcium hydroxide, CHX solution can be easily rinsed out of the canal with any irrigant; preferably, if the canal is irrigated with CHX immediately before root-filling to maximize the potential for substantive antimicrobial activity beyond that achieved by intracanal medication alone.

CONCLUSION

Under the conditions of the study, 1% Chlorhexidine Gluconate gel showed better antibacterial efficacy against *E. faecalis*. Vitapex paste with 30% Calcium hydroxide showed least inhibition of *E. faecalis*.

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