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Comparative Evaluation of Antimicrobial Efficacy of Calcium Hydroxide and Chlorhexidine Gutta Percha Points against *E. faecalis* and *S. mutans* - An *In Vitro* Study

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Abstract

Aim: To determine the antibacterial effectiveness of calcium hydroxide and chlorhexidine gutta-percha points against *Enterococcus faecalis* and *Streptococcus mutans*

Materials and methods: Eighteen extracted single-rooted human teeth were selected. After decoronation, root canals were prepared by using k files (Mani, Inc, Japan) up to master apical file size of forty. Following autoclave sterilization of these specimens, root canals were incubated at 37°C with *E. faecalis* and *S. mutans* for 24 hrs. Specimens were tested by inserting calcium hydroxide (Coltene/WhaledentInc.Germany), chlorhexidine (Roekoactivpoint, Germany) and conventional gutta-percha points (Endomax Guttapercha Points, Dento One Inc) for 1 hr, 3 hrs and 6 hrs respectively. Dentin chips collected after incubation were inoculated into agar plates and following an overnight incubation the colonies grown on agar plates were counted and interpreted as colony forming units. Results were tested statistically by using Friedman test for intragroup comparison at various time periods. One way Anova for intergroup comparison at different time periods. Overall two way Anova for comparison between the groups and the bacteria.

Results: When compared with conventional gutta percha points, calcium hydroxide and chlorhexidine gutta-percha points showed significantly lower colony forming units against *E. faecalis* and *S. mutans*

Conclusion: Chlorhexidine gutta percha points were more effective against *E. faecalis* and *S. mutans* than calcium hydroxide and conventional gutta-percha points.

Keywords: Activ gutta percha points; Antimicrobial activity; *E. faecalis*; *S. mutans*

Introduction

The fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent reinfection. Because of the complex anatomy of root canal systems biomechanical preparation procedures do not completely eliminate them. *Enterococcus faecalis* and *Streptococcus mutans* are the most commonly isolated microorganisms from infected root canals. *E. faecalis* is associated with persistent apical periodontitis and resists elimination from root canals [1]. *Streptococcus* species were reported to be one of the most relevant taxa in symptomatic apical periodontitis [2].

E. faecalis is found in 4 to 40% of primary endodontic infections [3]. Failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections [3].

S. mutans is another microorganism found in infected root canals associated with apical periodontitis [4]. *S. mutans* is relatively uncommon, it has been shown to be one of the most convenient microorganisms for use in the infected dentine model because of its ability to adapt to the laboratory setting, unlike strict anaerobic species [5].

Calcium hydroxide plays an important role in endodontics by its ability to induce hard tissue formation, moderate antibacterial action, and tissue dissolving capability [6]. Calcium hydroxide dressing may prevent root canal reinfection by interrupting nutrient supply to remaining bacteria [7].

Chlorhexidine has inhibitory effect on bacteria commonly found in endodontic infections acting against gram positive and gram negative microorganisms [8,9]. Its efficacy is based on the interaction between positive charge of the molecule and negatively charged phosphate groups on the bacterial cell wall. which allows chlorhexidine molecule to penetrate into bacteria with toxic effects [10].

In response to potential difficulties of conventional intra canal medicaments which were used in paste form and difficult to remove, sustained releasing devices were developed [11]. Such active gutta-percha points contain

substances with antimicrobial activity. Calcium hydroxide and Chlorhexidine gutta-percha points are among them [11].

The purpose of this study is to determine the antibacterial effectiveness of calcium hydroxide and chlorhexidine gutta percha points against *E. faecalis* and *S. mutans*.

Materials and Methods

Eighteen extracted straight single rooted human maxillary canines with single canal were selected for this study. Roots with resorption, fractures or open apices were excluded from this study. Soft tissue and calculus were removed from root surfaces with hand instrumentation and all teeth were stored in sterile saline solution until used.

Preparation of tooth samples

The teeth were decoronated at cement enamel junction to provide easy access to the canal space and to obtain a constant reference point for all instruments **(Figure 1)**. And roots of all specimens were standardized to length of 17 mm **(Figure 2)**. The contents of the canals were removed with barbed broach Initial apical patency of canal was checked with 10k-file **(Figure 3)** and canals were instrumented upto size 40k-file **(Figure 4)**. Subsequently canals were irrigated with 2.0 ml 2.5% sodium hypochlorite followed by 2.0 ml saline solution. Then apical seal was established with type II glass ionomer cement. External root surface of all specimens were coated with nail varnish to close dentinal tubules. And tooth samples were mounted on self-cure acrylic resin and are autoclave sterilized at 121°C for 15 minutes **(Figure 5)**. And they were divided into two groups with sample size of nine for each *Enterococcus faecalis* group and *streptococcus mutans* group. And they were subdivided into Conventional gutta-percha group. Calcium hydroxide group and CHX group with sample size of three each.

Figure 1 Decoronation at cemento enamel junction.

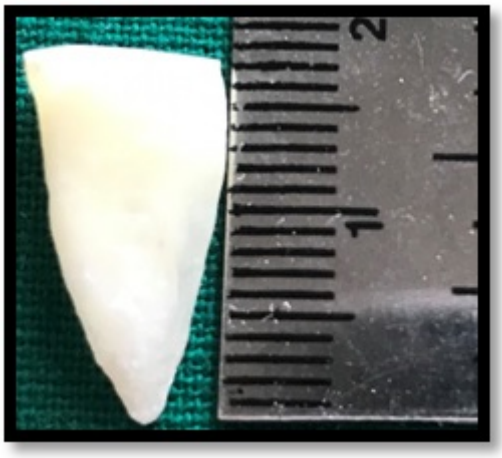


Figure 2 Standardization of root lengths to 17 mm.

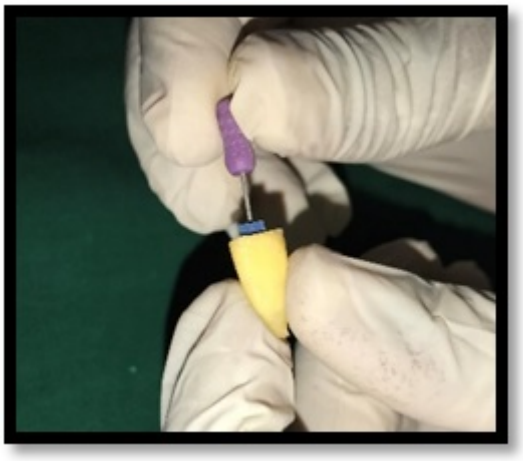


Figure 3 Initial apical patency with 10k file.

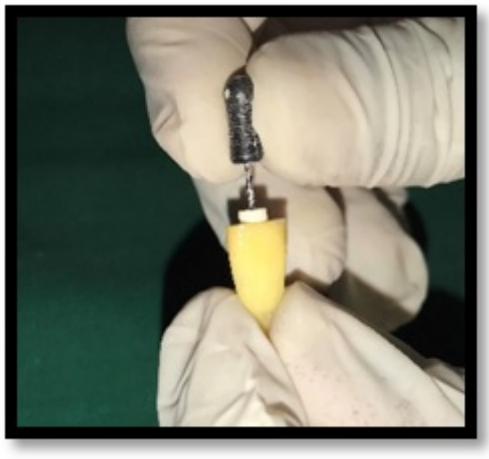


Figure 4 Master apical file of size 40k file.

Figure 5 Mounting of specimens on acrylic blocks.

Preparation of inoculum

All microbiological studies were conducted under aseptic conditions to prevent airborne bacterial contamination. *E. faecalis* and *S. mutans* stains were inoculated with the loop into peptone water and they were incubated for 24 hrs at 37°c under aerobic conditions till turbidity is obtained **(Figures 6 and 7).**

Treatment of tooth samples

0.5 ml of bacterial suspension was inoculated into mounted tooth samples of respective groups **(Figure 8)** and conventional gutta percha, Calcium hydroxide, and CHX were inserted into respective groups **(Figure 9)**. They were sealed coronally with GIC and the tooth samples were incubated at 37°C for 24 hrs.

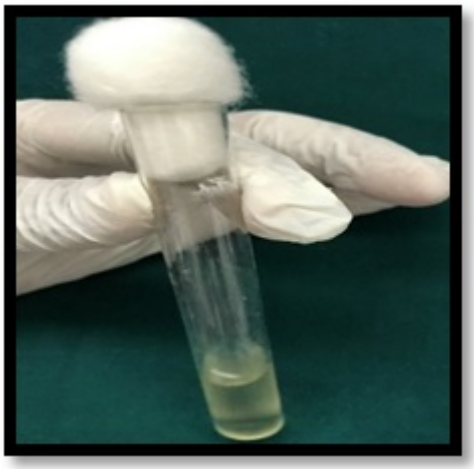


Figure 6 Peptone water with *E. faecalis* turbidity.

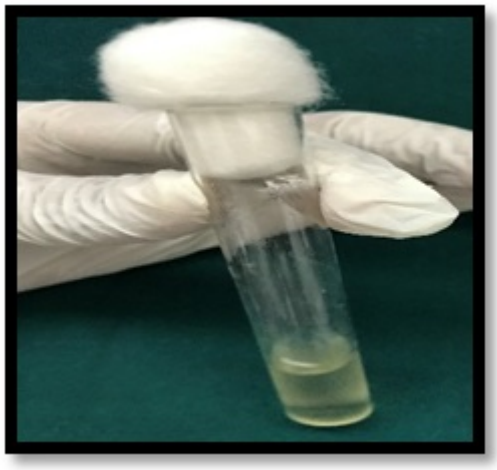


Figure 7 Peptone water with *S. mutans* turbidity.

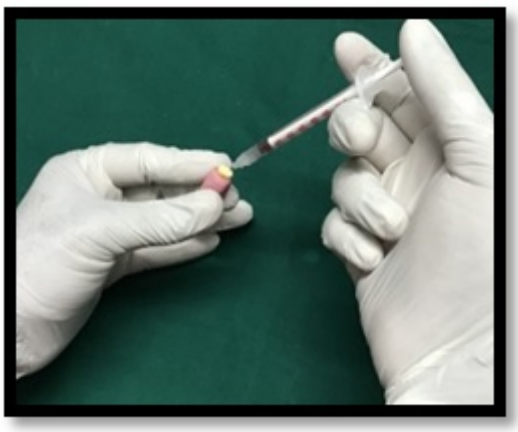


Figure 8 Inoculation of bacterial suspension into tooth samples.

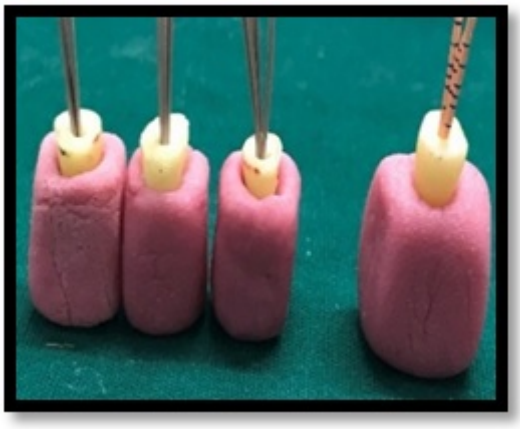


Figure 9 Insertion of gutta percha points into tooth samples.

Analysis of colony forming units

Gutta percha points were removed from respective groups and the gutta-percha points were washed with 1 ml of saline (0.9% sterile NaCl) solution each to recover as many bacteria as possible. Tooth samples were sectioned with disc and mandril **(Figure 10)** than dentin was scraped with spoon excavators along the canal walls and were inoculated into test tubes containing peptone water **(Figure 11)**. Test tubes containing peptone water is incubated for 24 hrs at 37°C aerobically till turbidity is obtained. Than the inoculum of respective groups was inoculated into agar plates and incubated for 24 hrs at 37°C for colony forming units.

Statistical Analysis

Data was analysed using SPSS version 22, Descriptive statistics Friedman's test for intragroup comparison at various time periods. One way Anova for intergroup comparison at different time periods. Overall two way Anova for comparison between the groups and the bacteria **(Table 1 and Graph 1)**.

Table 1 Intragroup comparison of various clinical parameters.

Group	I hour				3 hours				6 hours				p value	p value
	Enterococcus	*Streptococos*	*Enterococcus*	*Streptococos*	*Enterococcus*	*Streptococos*	*Enterococcus*	*Streptococos*	*Enterococcus*	*Streptococos*	*Enterococcus*	*Streptococos*	Bacteria A at various time periods	Bacteria B at various time periods
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Chlorhexidine	7.167	0.3055	7.733	0.3215	5.8	0.4359	5.533	0.8386	3.1	0.2	3.2	0.2	0.05*	0.05*
calcium hydroxide	7.633	0.3512	7.9	0.3606	7.167	0.3055	7.267	0.5508	6.6	0.4359	7.167	0.3055	0.05*	0.097 NS
Conventional	8.067	0.2082	8.133	0.3512	8.1	0.3606	8.067	0.5508	8	0.3606	7.933	0.3512	1 NS	0.717 NS
Intergroup comparison p value	0.027*		0.001**		<0.001**		0.416 NS		0.009**		<0.001**		<0.001**#	

p value <0.05 is considered as statistically significant; * Statistically Significant (p<0.05) **; Statistically Highly Significant (p<0.01); NS - Not Signifcant (p>0.05); # - overall two way anova p value (comparisons between the groups and the bacteria)

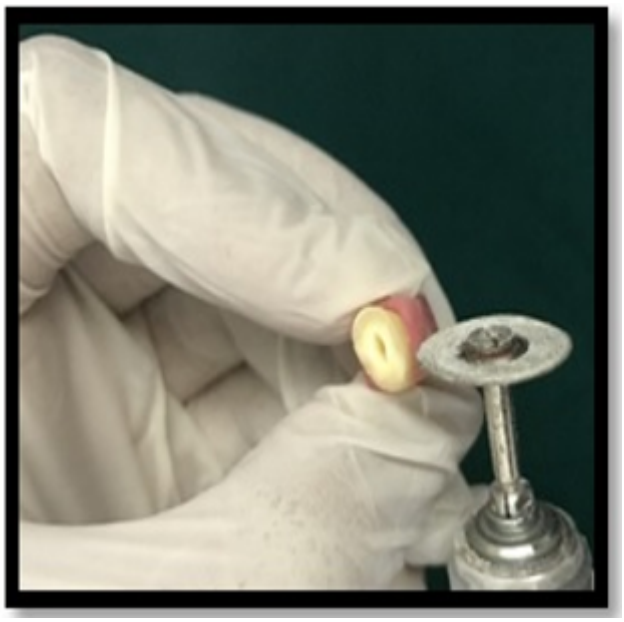


Figure 10 Sectioning with disc mandrel.

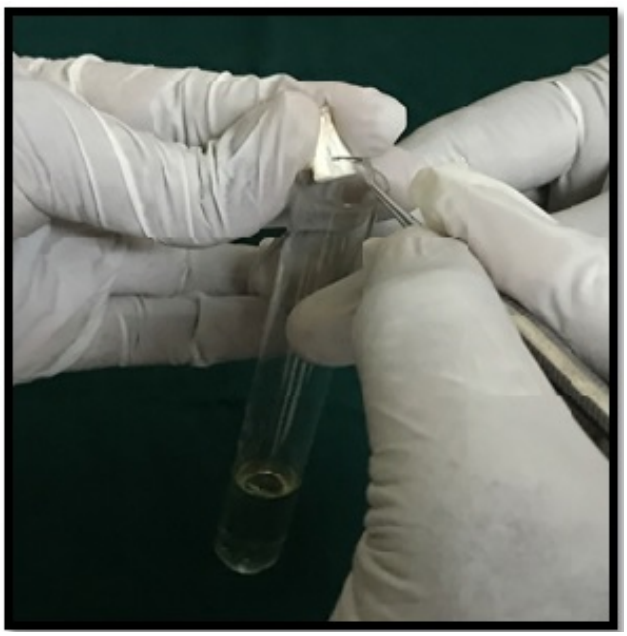
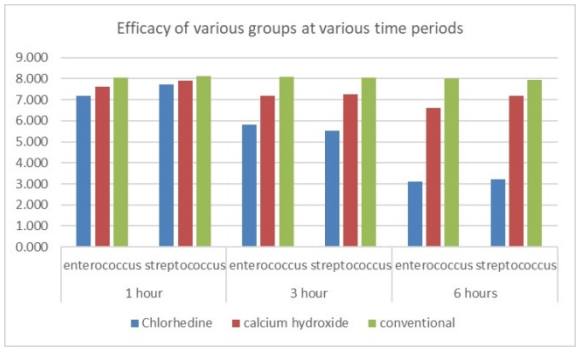


Figure 11 Dentin scraping with spoon excavator.



Graph 1 Zone of inhibition of chlorhexidine, calcium hydroxide and conventional gutta-percha points at different time periods. p value <0.05 is considered as statistically significant.

X-axis represents time intervals and Y axis represents zone of inhibition of *E. faecalis* and *S. mutans* with chlorhexidine gutta percha points, calcium hydroxide gutta percha points and conventional gutta percha points.

Results

The colony forming units of *E. faecalis* and *S. mutans* after treatment with $Ca(OH)_2$, CHX and conventional gutta percha points were shown **(Figures 12-17)**.

There is significance decrease in colony forming units with increase in time interval upto 6 hrs in $Ca(OH)_2$ and CHX groups.

Table 1 shows the mean and SD of the zone of inhibition at various time periods.

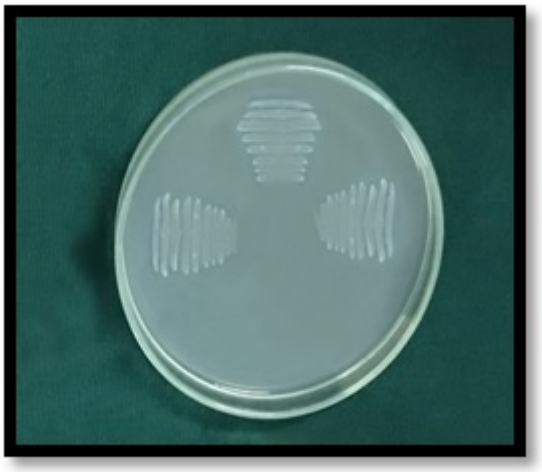


Figure 12 Conventional gutta percha group showing colony forming units at 1 hr, 3 hrs and 6 hrs.

Intragroup comparison at various time periods was done using Friedman test showed there is a statistically significant differences present between the mean values at various time periods in chlorhexidine group calcium hydroxide and conventional group which showed that chlorhexidine is more effective for *Enterococcus.*

Figure 13 Calcium hydroxide group showing colony forming units at 1 hr, 3 hrs and 6 hrs.

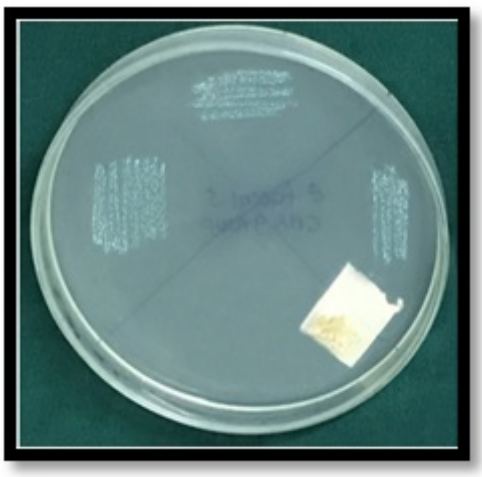


Figure 14 Chlorhexidine group showing colony forming units at 1 hr, 3 hrs and 6 hrs.

Intragroup comparison at various time periods was done using Friedman test showed there are statistically significant differences present between the mean values of mean zone of inhibition at various time periods in chlorhexidine calcium hydroxide & conventional group for *streptococcus* which showed that chlorhexidine is more effective for *streptococcus.*

Intergroup comparison is done by one way Anova showed statistically significant difference at all the time periods in both bacteria.

Overall two way Anova showed that there is statistically significant difference in mean zones of inhibition at various time periods in both the bacteria and the chlorhexidine is more effective than others($p < 0.001$).

Figure 15 Conventional gutta percha group showing colony forming units at 1 hr, 3 hrs and 6 hrs.

Figure 16 Calcium hydroxide group showing colony forming units at 1 hr, 3 hrs and 6 hrs.

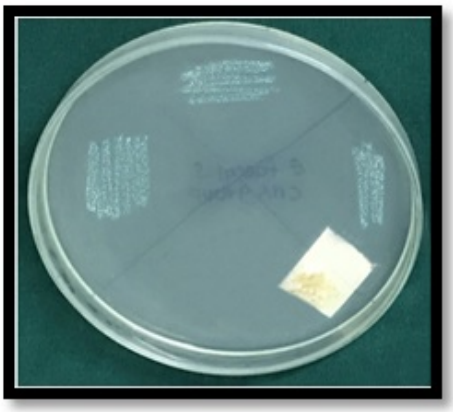


Figure 17 Chlorhexidine gutta percha points showing colony forming units at 1 hr, 3 hrs and 6 hrs.

Discussion

Endodontic therapy is based on nonspecific elimination of intra radicular microorganisms. Many studies have shown the importance of intracanal dressings for elimination of bacteria that cannot eliminated by biomechanical preparation. Several antimicrobial agents have been tested for their ability to eliminate *E. faecalis* and *S. mutans* from the root canal system. These include both irrigants, such as sodium hypochlorite, hydrogen peroxide, chlorhexidine digluconate and iodine compounds, as well as interappointment dressings, such as calcium hydroxide, chlorhexidine gluconate, camphorated phenol and mixed antibiotic-steroid combinations. This study investigated the antimicrobial potential of three different medicaments i.e., calcium hydroxide, chlorhexidine and conventional gutta-percha points against *E. faecalis* and *S. mutans*.

This study mimicked clinical conditions by using human teeth instead of bovine teeth. *E. faecalis* and *S. mutans* were chosen as test microorganisms in this study because they are most common dental pathogens [12]. *E. faecalis* has been shown to exhibit widespread genetic polymorphisms. It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which help it bind to dentin.

E. faecalis is small enough to proficiently invade and live within dentinal tubules. It has the capacity to endure prolonged periods of starvation until an adequate nutritional supply becomes available. Once available, the starved cells are able to recover by utilizing serum as a nutritional source. Serum, which originates from alveolar bone and the periodontal ligament, also helps *E. faecalis* bind to type I collagen [3].

S. mutans has been detected in root canal infections and was also reported to be a strong biofilm producer [4,13], helping the bacteria to adapt and persist in root canals. The results of this study clearly indicated that $Ca(OH)_2$ was not sufficient for the complete elimination of *S.mutants* from root canals.

In this study dentin chips were directly collected into peptone water and not in saline or transport medium which helps the growth of *E. faecalis* and *S. mutans* this method was modification of previous study done in which they used RTF or VMGAIII as medium [14].

This study demonstrated that the number of CFU's were less in chlorhexidine impregnated gutta percha points than $Ca(OH)_2$ guttapercha points. This relative inefficacy of $Ca(OH)_2$ against *E. faecalis* was consistent with previous studies [15-17]. $Ca(OH)_2$ was effective in the superficial dentine compared with the deeper layers [12]. The reason for this result was reported to be the superficial exposition of microorganisms to lethal levels of hydroxyl ions only at the tubule orifice [7].

One mechanism that can explain the *in vivo* antibacterial activity of $Ca(OH)_2$ is to absorb CO_2 in the root canals which is essential for bacteria such as *Capnocytophagia*, *Eikenella* and *Actinomyces sps*, and is provided by the bacteria such as *Bacteriods*, *Fusobacterium*, *Porphyromonas* [18,19].

Chlorhexidine in gel formulations have important properties such as low cytotoxicity to periapical tissues viscosity that

keeps the active agent in contact with root canal walls, dentinal tubules and water solubility [20,21]. But in the present investigation chlorhexidine impregnated gutta percha points were used which showed antibacterial efficacy with increased time intervals upto 6 hrs against *E. faecalis* and *S. mutans*. This result agrees with those of others eventhough those studies utilized chlorhexidine in liquid or gel forms at different concentrations [22-24].

Hence the results of present investigation shows that chlorhexidine impregnated gutta-percha points were more effective against *E. faecalis* and *S. mutans* when compared with calcium hydroxide and conventional gutta percha points.

Conclusion

Within the limitation of this *in vitro* study it can be concluded that there is significant difference in the antimicrobial activity of Calcium hydroxide points and Chlorhexidine impregnated gutta percha points (Activ points) against *Enterococcus faecalis* and *Streptococcus mutans*. Chlorhexidine impregnated gutta perch points are more effective than Calcium hydroxide gutta percha points and conventional gutta percha points.

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