

# A Comparative Evaluation of Antibacterial Efficacy of ‘Activ Points’ And ‘Combi Points’ as Intra-Canal Medicaments Against *Enterococcus faecalis*: An *Ex Vivo* Study

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## Abstract

**Background:** *Enterococcus faecalis* has been found to be one of the most predominant bacterial species associated with failed endodontic cases. The ability of this microbe to form biofilms; penetrate into dentinal tubules; survival in low pH, high salinity and high temperatures and resistance to many intracanal medicaments, makes it one of the most resistant pathogen of all the root canal flora. Studies have shown that chlorhexidine is relatively more effective against *E. faecalis* as compared to other intra canal medicaments. But its placement in solution form or gel form up to the root apex is not only difficult but uncertain as well. The purpose of this study was to compare the antimicrobial efficacy of chlorhexidine releasing Activ points™ chlorhexidine and calcium hydroxide releasing Combi points™; with that of standard 2% chlorhexidine solution.

**Materials and Methods:** Thirty McConkey agar plates were inoculated with *Enterococcus faecalis* and were divided into three groups. Test specimens (paper points soaked in 2% chlorhexidine solution chlorhexidine releasing Activ points™ and chlorhexidine and calcium hydroxide releasing Combi points™; 20 each) were placed in the plates and the zone of inhibition was measured around the specimen after 24 and 48 hrs. The results so obtained were subjected to statistical analysis.

**Results:** Statistically significant difference was found in terms of zone of inhibition in between the three groups greatest being for Combi points™.

**Conclusion:** The findings of the present study suggest that Combi points™ and Activ points™ can be used safely as an alternative to 2% chlorhexidine solution as intra canal medicament with the additional advantage of availability of these points in ISO standard sizes easy placement up to the root apex and easy retrieval from the root canal system.

*Key words:* Activ points; Chlorhexidine; Combi points; *Enterococcus faecalis*

## Introduction

Bacteria and their by-products are considered the primary etiological agents of necrotic pulp and apical periodontitis. Accordingly, the aim of root canal treatment is elimination of infection from the root canal system. With advancing technology, better understanding of root canal anatomy, and improved materials, root canal therapy is achieving an increasingly high overall success rate [1]. However, there have been some cases, in which the treatment has followed the highest technical standards and yet failure has been reported [2]. The chances of favorable outcome with root canal treatment are significantly higher if all microbes are eradicated effectively before the root canal system is obturated. However, if the microorganisms persist in the root canal system, there is a higher risk of treatment failure [2].

Studies have shown that certain bacteria form biofilms and thus render themselves resistant to removal from root canal system. Some of these microbes also penetrate into the dentinal tubules and thus escape the bio-mechanical preparation procedure. It is also needful to realize that some parts of the root canal system may remain untouched during cleaning and shaping procedure, regardless of the technique and instruments employed. These untouched areas may contain bacteria and necrotic tissue substrate. Such areas may be responsible for large number of endodontic failures [2].

*Enterococcus faecalis* has been found to be one of the most predominant bacterial species associated with failed endodontic cases [3]. Its prevalence in failure root canal cases ranges from 24-77% [4] probably owing to its ability to form

biofilms; establish monoinfection; get converted into Viable but Non Cultivable state (VBNC); ability to survive low pH, high salinity and high temperatures; Gene encoded antibiotic resistance etc. Eradication of *E. faecalis* from the root canal system may perhaps form one of the most rational steps taken towards increasing the success rate of endodontic therapy.

To reduce the number of microbes, many materials have been used as intracanal medicaments such as calcium hydroxide, camphorated paramonochlorophenol, iodine-potassium iodide, chlorhexidine, formocresol etc [5-8]. Among these medicaments, calcium hydroxide is effective against most of the root canal microbial isolates [9] except *E. faecalis* which can tolerate its high alkalinity and hence survives [10].

Studies have shown that chlorhexidine alone or a combination of chlorhexidine and calcium hydroxide are relatively more effective against *E. faecalis* as compared to other intra canal medicaments [10,11]. These medicaments are either used in paste or gel form and so are quite difficult to place in root canal system up to the apex and also difficult to retrieve [12,13].

Recently, medicated gutta percha points releasing Chlorhexidine have been introduced to be used as intra canal medicament (Activ Points, ROEKO, Germany). Also, chlorhexidine and calcium hydroxide releasing ‘Combi points’ (ROEKO, Germany) are being studied upon for their efficacy against *E. faecalis*. These points are easy to place in root canal system up to the root apex, easy to retrieve and are available in ISO sizes [14].

The present in-vitro study was undertaken to evaluate the

antimicrobial efficacy of these Chlorhexidine releasing 'Activ Points' and Chlorhexidine and Calcium hydroxide releasing 'Combi points' against *E. Faecalis* and compare with that of conventional 2% chlorhexidine solution.

### Materials and Methods

A standard bacterial strain of *E. faecalis* was used in this study. *E. faecalis* (ATCC 47077) was obtained from Microbial Type Cell Culture (MTCC) (Institute of microbial technology, Chandigarh).

*E. faecalis* was maintained on blood agar plates. Three to five isolated colonies from each plate were grown at 37°C in Tryptic Soy broth for 2 hrs at 300 RPM. The turbidity of each actively growing culture was adjusted to that of 0.5 McFarland standards. A sterile cotton swab was dipped into the adjusted bacterial suspension. Ten McKonkey agar plates were then inoculated by streaking the swab over the entire sterile agar surface. These agar plates were divided into three groups, depending on the test specimen used viz. Paper point soaked with 2% Chlorhexidine solution were designated as Group I; Activ points' (Roeko, Germany) designated as Group II and 'Combi points' (Roeko, Germany) were designated as Group III.

Each McKonkey agar plate was divided into two halves so that for each group 20 test agents could be used. The two test agents of same group were placed in each McKonkey agar plate at a distance. Then the McKonkey agar plates were incubated for 24 hours at 37°C aerobically. Evidence of growth on the plates was observed and the zones of inhibition were measured using digital vernier caliper.

### Observations and Results

The zones of inhibition, as measured by a digital vernier caliper are tabulated in Table 1. After 24 hours, no zone of inhibition was seen in 18 out of 20 samples for Group I (Figure 1). Zone of inhibition was seen in all the samples of Group II (Figure 2) and III (Figure 3). After 48 hours, some

**Table 1.** Zone of inhibition (in mm) measured for all the three groups.

S No.	Group I		Group II		Group III	
	24hr	48 hr	24hr	48 hr	24hr	48 hr
1.	0	2.90	3.10	6.17	5.15	7.80
2.	0	2.12	3.11	4.26	6.20	7.68
3.	0	2.80	5.02	5.87	5.66	7.75
4	0	2.51	5.05	5.71	5.90	7.90
5	0	2.65	4.42	6.67	6.27	7.95
6	0	2.70	5.01	6.86	6.34	7.11
7	0	2.45	5.26	6.29	5.80	7.46
8	0	3.13	5.05	6.70	6.67	7.84
9	0	2.67	5.01	5.19	6.80	7.74
10	0	3.15	5.03	5.30	5.70	7.93
11	1.20	2.80	4.90	6.25	6.45	7.68
12	0	2.90	5.11	6.40	5.60	6.96
13	1.1	3.10	4.60	6.28	5.98	7.56
14	0	3.20	5.24	5.60	6.18	7.44
15	0	2.65	3.90	5.88	6.29	7.70
16	0	2.11	4.20	6.12	6.50	6.84
17	0	3.12	4.10	5.50	6.69	7.25
18	0	3.67	4.78	6.18	5.70	7.49
19	0	2.78	5.12	5.90	5.95	7.72
20	0	2.85	4.89	6.26	6.11	7.10



**Figure 1.** No zone of inhibition seen in Group I sample.



**Figure 2.** Zone of inhibition seen in Group II sample.



**Figure 3.** Zone of inhibition seen in Group III sample.

zone of inhibition started appearing in samples for Group I while an increased zone of inhibition was observed in all the samples of Group II and III. The mean zone of inhibition of each group was calculated after 24 hours and 48 hours for all the three groups at 24 and 48 hours respectively. Statistical analysis was performed using paired T-test for the mean zones of inhibition between the different groups. The significance value was set at  $p < 0.05$ .

When compared, a statistically significant difference was

found between Group I and II, with latter performing better than former ( $p < 0.05$  = significant), both at 24 and 48 hours. Also, when Group III was compared to Group II and Group I, it performed better than them, both at 24 and 48 hours and this was found to be statistically significant ( $p < 0.05$ ).

### Discussion

Retreatment of endodontic failure cases is perhaps the biggest challenge an endodontist comes across. Amongst the various reasons for an endodontic failure, the persistence of resistant bacterial species is a major contributing factor [1,15]. Of all the bacteria isolated from root canals, *E. faecalis* has been found to be most prominent in both primary treatment and retreatment groups [1,4,16,17].

These bacteria usually survive both chemomechanical preparation as well as intracanal medication and reside in the canal long after completion of therapy as well. The probable reason for this is the inefficiency of the conventional antimicrobial agents used during endodontic therapy [1].

The need of the hour is thus to use the antimicrobial agents which are effective against *E. Faecalis*. Siren et al. in their study confirmed the effectiveness of Chlorhexidine as an intracanal medicament in cases where *E. Faecalis* was suspected [18]. Similar conclusion has also been reported by Gomes et al (2003) and Menezes et al (2003), Lui et al. [19] and Singh et al. [20]. However researches have also not negated the combined use of Chlorhexidine and Calcium Hydroxide as intra canal medicament to be used against *E. Faecalis* [21].

The only apprehension in use of Chlorhexidine which is available in liquid/gel form is the precision with which it can be placed up to the apical end of root canal system so as to achieve its maximum beneficence. Similar is the problem with placement of Calcium Hydroxide paste. Recently, 'Chlorhexidine releasing' points 'Active points' and 'Chlorhexidine and Calcium Hydroxide combined releasing' points 'Combi points' have been introduced, the manufacturers of which claim the consistent and effective release of respective medicaments from these points which are available in ISO sizes from 15-40.

The aim of our study was to compare the antibacterial

efficacy of '2% Chlorhexidine solution (paper point soaked in 2% Chlorhexidine solution)', 'Activ points' and 'Combi points' against *E. faecalis*. Culture media inoculated with Mcfarland standard of *E. faecalis* were placed in these culture plates. At consistent incubation of 37°C, the zone of inhibition was measured in all the test samples by the use of a digital vernier caliper at a span of 24 hrs and 48 hrs. 'Activ points' showed greater zone of inhibition as compared to Chlorhexidine solution at both 24 hrs and 48 hrs which was found to be statistically significant; thus proving their superior efficacy. However, 'Combi points' showed the maximum zone of inhibition, more than 'Activ points' and 2% Chlorhexidine solution. These results are not in accordance with some of the previous studies [22] wherein chlorhexidine alone has shown superior performance in comparison to the combination. This can be explained probably by more consistent release of both the medicaments from the 'Combi points. An analysis on similar patterns has been performed by Tanomaru et al. wherein the efficiency of Activ points was proved against several other microbes using the agar diffusion test [23].

However, agar diffusion test has its own drawbacks for the fact that it cannot really match up with the actual clinical conditions and this forms a limitation of this study. But, the fact that this test is one of the most easily available methods to the researchers all across the globe cannot be overlooked [24].

The availability of these points in ISO standard sizes ensures their accurate placement up to the working length. Their placement pattern being similar to gutta percha points allows easy placement and retrieval from the root canal system. The radio-opacity of these points provides an extra benefit in terms of verification of their extent as can be seen on a radiograph.

Owing to all such clinically effective properties and of course the proven biological effects against *E. faecalis*, it can be concluded that both "Activ points" and 'Combi points' may prove to be quite efficacious in primary infected cases as well as reinfected cases where *E. faecalis* is a major microbial obstacle that needs to be dealt to ensure successful endodontic therapy. However, a more detailed clinical analysis needs to be done before the combination therapy can be advised as a superior modality to use of Chlorhexidine alone for combating *E. faecalis*.

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