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AN EVALUATION OF ANTIBACTERIAL EFFICACY OF CONVENTIONAL INTRACANAL MEDICAMENTS AND MEDICATED GUTTA PERCHA POINTS AT DIFFERENT TIME INTERVALS AGAINST *E. FAECALIS*: AN IN-VITRO STUDY

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ABSTRACT

Background: In endodontic failure cases *Enterococcus faecalis* is one of the most predominant bacterial species detected. The capability of this microorganism to render itself resistant in the root canal system makes it one of the most resistant pathogen of all the root canal flora. The aim of this study was to evaluate the Antibacterial efficacy of Conventional Intracanal Medicaments and Medicated points against *E. faecalis* at different time intervals. **Materials and Methods**: Tested materials had two groups (I & II) & group C was kept as control group. Group I consisted of conventional intracanal medicaments and Group II had medicated points. Conventional gutta percha points were taken as control group. Group I was further divided into subgroup A & B (subgroup A: paper discs soaked in calcium hydroxide medicament & in subgroup B paper discs were soaked in 2% chlorhexidine digluconate solution). Similarly group II was divided into A & B (Subgroup A: calcium hydroxide releasing points, Subgroup B: chlorhexidine releasing Activ point) were used. Conventional gutta percha points were taken as control group and designated group C. The zones of inhibition against these tested materials were measured after 24, 48, 72 hrs & 7 days using a digital vernier caliper. The results obtained were subjected to statistical analysis. **Results**: The difference was found in terms of zone of inhibition in between the tested groups. The size of inhibition zone against medicated gutta percha points increased with time. **Conclusion:** Results showed that medicated points can be used safely as an alternative to conventional intracanal medicament.

KEYWORDS: Calcium Hydroxide points; Chlorhexidine Activ points; Enterococcus faecalis, Intracanal Medicaments.

INTRODUCTION

Microorganisms and their by products are considered the primary etiological agents in the pulpal and periapical diseases. The root canal infection is polymicrobial, typically dominated by anaerobic microbes.[1] The main objective of the endodontic treatment is eradication of infection from the root canal system.[2] Recent advancements in field of endodontics has made the understanding of root canal anatomy better and with the use of superior instruments & materials, root canal treatment is attaining high success rate.[3] But still failure of the root canal therapy has been reported in many cases even with highest technical standards.

The primary reason for unfavorable outcome is due to persistence of microbes in the root canal system. Some parts of the root canal system such as lateral and accessory canals, isthmi, cul-de-sac and apical deltas may be left uninstrumented during biomechanical preparation, regardless of the method and instruments employed. These uninstrumented areas may possibly hold bacteria and necrotic tissue leading to endodontic

failure. On the other hand several studies have revealed that certain bacteria render themselves resistant to removal from root canal system. Some microbes can also make a way into the dentinal tubules and thus escape during cleaning and shaping procedures.[4]

Enterococcus faecalis is a gram-positive facultative anaerobic bacterium, commonly isolated from root canals of teeth with endodontic failure.[5] Its prevalence in primary endodontic infection is 40% and in failure root canal cases ranges from 24-77%.[6] Thus *Enterococcus faecalis* is one of the most predominant bacterial species associated with unsuccessful endodontic cases possibly due to its ability to endures prolonged period of nutritional deprivation; binds to dentin & proficiently invades dentinal tubules.[7] It survives in a low pH, high salinity & temperatures and gene encoded antibiotic resistance etc.

Complete elimination of *E. faecalis* from the root canal system is one of the most challenging task of endodontic therapy. For this many materials have been used as intracanal medicament such as calcium hydroxide, camphorated paramonochlorophenol, iodine potassium iodide, chlorhexidine, formocresol etc.[8] Calcium hydroxide intracanal medicament is successful against most of the root canal bacteria[9] except *E. faecalis* as it can bear its high alkalinity and hence survives.[10] Studies have revealed that chlorhexidine alone or in combination with calcium hydroxide are comparatively more effective against *E. faecalis* as compared to other intra canal medicaments.[10, 11]

As well as these intra canal medicaments are present in liquid/powder or gel form. A great amount of precision is needed to place them to apical end of the root canal to achieve its maximum benefits. In addition to placement, the removal of calcium hydroxide and chlorhexidine from the root canal without leaving any remnants behind is a time consuming and cumbersome procedure.[12] With the aim to overcome these short comings & provide better antimicrobial action for longer duration, impregnated points have been manufactured including substances such as calcium hydroxide and chlorexidine. These points can be easily placed in root canal system up to the apex and easily retrieved thus providing a uniform antimicrobial action throughout the canal.[13] But a very limited information is present about the durability & efficiency of these medicated points.

Thus considering all these factors this in vitro study was aimed to evaluate the Antibacterial efficacy of Conventional Intracanal Medicaments and Medicated points against *E. Faecalis* at different time intervals.

MATERIAL AND METHOD

The calcium hydroxide powder (PrevestDenpro), 2% chlorexidine digluconate solution (PrevestDenpro), calcium hydroxide points (Coltene) & chlorexidine activ points (Roeko) were used as test groups. Conventional gutta percha points (Dentsply maillefer) were taken as control to check their antibacterial efficacy against *E.faecalis.*

Tested materials were divided into two groups as follows:

Group I:- consists of conventional intracanal medicaments.
Subgroup A: paper discs soaked in calcium hydroxide solution.
Subgroup B: paper discs soaked in 2% chlorhexidine digluconate solution.

Group II:- had medicated points.
Subgroup A: calcium hydroxide releasing points.
Subgroup B: chlorhexidine releasing Activ point.
Subgroup C: had conventional gutta percha points as control group..

The microbial procedures were performed in the Biosafety Cabinet. Pure culture of *E. faecalis* (ATCC 29212) was grown in Brain Heart Infusion broth (BHI) for 24 hours at 37˚C. (fig 1 & 2).

The turbidity of actively growing culture was adjusted acc. to 0.5 McFarland standards.

Figure 1: Microbial strain *Enterococcus Faecalis* (ATCC 29212)

Figure 2: Bacterial colonies in petridishes

In the present study the method opted for antibacterial testing was agar diffusion method. Mueller-Hinton Medium was prepared and poured into six petri dishes. Petri dishes were allowed to cool down for solidification of medium. Then 50 µl of bacterial broth suspension was spread on to the Petri dishes containing Mueller Hinton agar medium using sterile glass spreader.

The paper discs were soaked with two different intra canal medicaments as used in group I and were placed in three petri dishes. (Fig3).

Subsequently, in the other three petri dishes one cone from each test materials in group II & the control group i.e group C were aseptically transferred onto the inoculated plates using sterile forceps (Fig 4).

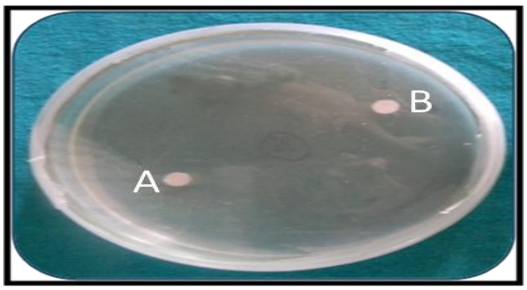


Figure 3: Group I: Petridishes containing medicated discs

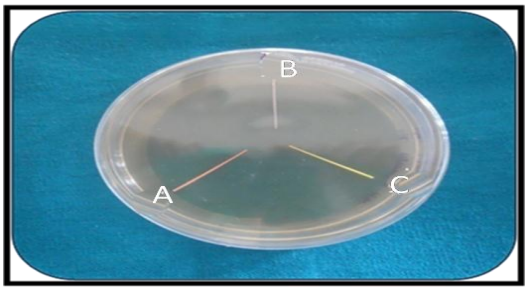


Figure 4: Group II: Petridishes containing GP

All the Agar plates were placed in the incubator at 37˚ C. After incubation Diameters of zones of inhibition were measured at 24, 48, 72 hr & 7 days with Digital Vernier caliper. (fig 5 & 6) The values obtained were stastically analysed and mean and standard deviation values among different groups at different time intervals were calculated.

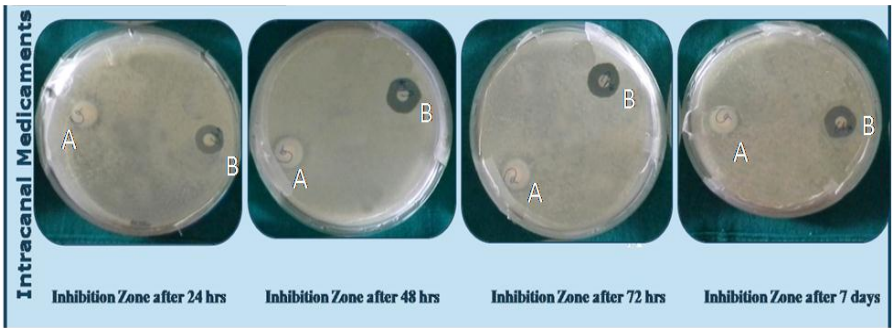


Figure 5: The inhibition zone around conventional intra canal medicaments. (Group I)

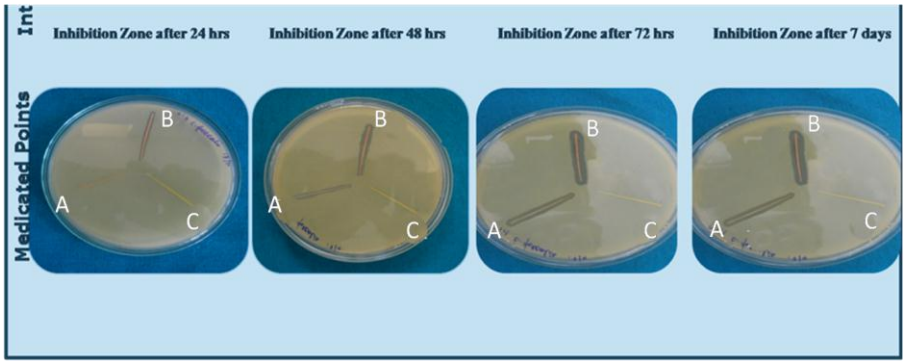


Figure 6: The inhibition zone around medicated gutta percha points. (Groups II)

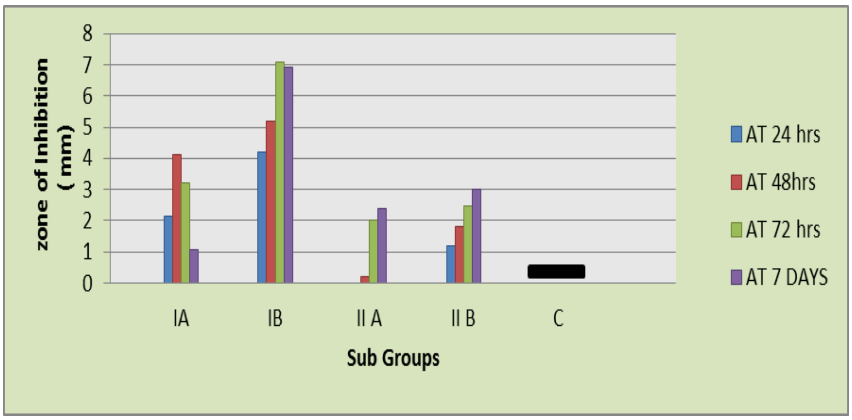
STATISTICAL ANALYSIS AND RESULTS

It was seen that the mean zone of inhibition had a greater diameter in chlorhexidine containing petridishes, which also increased in size at all time intervals (24 hours, 48 hours, 72 hours and 7 days) as depicted in Table 1 & Graph 1.The conventional calcium hydroxide used as an intra canal medicament had the antimicrobial activity for a shorter duration as the zones of inhibition against *E. faecalis* began to decrease in size at 72hrs, where as a slight decrease in size in inhibition zone in 2% CHX digluconate was seen at 7 days.

When the efficacy of chlorhexidine and calcium hydroxide in the form of medicated gutta percha points was compared, it was observed that chlorhexidine containing active points had early onset of action with the zone of inhibition appearing at 24 hours as compared to calcium hydroxide impregnated GP points in which the zone of inhibition appeared at 48 hours. The zones of inhibition increased in size against both medicated gutta percha points used till 7 days. The conventional gutta percha points used as control group did not showed any antibacterial efficacy.

Table 1: Inter group comparison of mean & standard deviation of all the subgroups against *E. faecalis* at different time intervals.

Sub Group	N	At 24 hr. (in mm) Mean	±SD	At 48 hr. (in mm) Mean	±SD	At 72 hr. (in mm) Mean	±SD	At 7 Days. (in mm) Mean	±SD
IA Conventional $Ca(OH)_2$	9	2.15	0.97	3.90	0.18	4.10	0.87	1.07	0.98
IB Conventional 2% CHX digluconate	9	4.2	1.03	5.20	1.10	7.10	1.03	6.90	1.08
IIA $Ca(OH)_2$ impregnated GP	9	0.00	0.00	1.44	0.21	2.00	0.10	2.40	0.08
IIB CHX Active points	9	1.17	0.16	1.79	0.12	2.48	0.17	3.00	0.21
C Conventional GP	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00



Graph 1: Inter group comparison of mean zones of inhibition of the subgroups against *E.faecalis* at different time intervals

Therefore the results of the present in vitro study indicate that chlorhexidine is a possible alternative to calcium hydroxide as an intracanal medicament and the medicated gutta percha points are always better than the conventional intracanal medicament as they possess antibacterial activity for a longer duration.

DISCUSSION

The main objective of the endodontic treatment is eradication of infection from the root canal system but irregularities of root canal system may harbor pulp remanants, debris, bacteria in spite of thorough biomechanical prepration.[14] Therefore it is extremely desirable that instrumentation should be accompanied by irrigation & intracanal medicaments for the eradication of bacteria to achieve complete sterilization of root canal.[15] In this present study E.faecalis was selected as the test organism because it is a facultative organism that is nonfastidious, easy to grow and proficiently and quickly colonizes in the dentinal tubules.[16] It has been used widely in endodontic research work because it is the major organism detected in post treatment diseases due to the high level of resistance to a wide range of antimicrobial agents.

E. faecalis overcomes the various challenges and continue to exist within the root canal system in several ways: (Isabelle Portenier et al).[17]

1. It possesses serine protease, gelatinase, and collagen-binding protein (Ace), which helps it bind with dentine.

2. It is small enough to proficiently invade and live within dentinal tubules.

3. 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.

4. *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide even for over 10 days. E. faecalis is able to form a biofilm that helps it to resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials.

The agar diffusion test used in the present study is one of the most commonly used method for estimation of the anti-microbial action of endodontic materials.[18] It allows comparisons of the filling materials against the test microorganisms, signifying which material has the talent to remove bacteria in the local micro environment of the root canal system.[19] The present study used Brain heart infusion as the culture media, since these media are easily available and frequently used media for *Enterococcus faecalis.*[20]

$Ca(OH)_2$ is bactericidal and buffers the residual tissue debris in the root canal system. Estrela et al claimed that $Ca(OH)_2$ inhibits bacterial enzymes by means of hydroxyl ions of the bacteria's cytoplasmic membrane, generating the antibacterial effect. For calcium hydroxide to act effectively as an intracanal dressing, it should occupy all the pulp space and diffuse into areas inaccessible to instruments. Its high pH (around 12.5) has a damaging effect on cell membranes and protein structure of the microorganisms.[21]

Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent that has been used as an effective intracanal medicament.[22] CHX molecule penetrates into the bacteria due to increase in permeability of the cell wall. The most common water soluble preparation, CHX digluconate which at physiologic pH, dissociates and releases the positively charged CHX component. At low concentration (0.2%), CHX is bacteriostatic, causes potassium and phosphorous, to leak out of the cell. On the other hand, at higher concentration (2%), CHX digluconate is bactericidal, it precipitates cytoplasmic contents, which results in cell death.[23]

These intracanal medicaments are present in liquid/powder or gel form. A great amount of precision is needed to place them to apical end of the root canal to achieve its maximum benefits. In addition to placement, there removal from the root canal without leaving any remnants behind is a time consuming and burdensome procedure.[12] With the aim to overcome these short comings & provide better antimicrobial action for longer duration, gutta-percha points have also been introduced that contain a high percentage of calcium hydroxide (Coltene Germany) and chlorhexidine (Activ Point, Roeko). This permits a simple placement of the medicament within the canal space between appointments. Once the medicament has leached out, the point is no longer useful and must be removed.

These gutta-percha points contain substances with antimicrobial activity that is released when they come in contact with moisture (Distler & Petschelt 1997).[24] The advantages of these materials are that there is no mixing procedure, easy to apply and remove, leaves no residue and the root canals could be filled till the apex.[25]

This study demonstrated that there was significant difference in the antimicrobial activity of Conventional intracanal medicaments (Calcium Hydroxide & 2% Chlorhexidine Digluconate) and Medicated gutta percha points (Calcium hydroxide points and Chlorhexidine Activ points) against *Enterococcus faecalis.*

The chlorhexidine activ points & $Ca(OH)_2$ points showed an increase in antibacterial activity with time, where as actibacterial activity of conventional intracanal medicaments was only for shorter duration.

The intracanal medicaments has an abrupt action and becomes inactive after some time whereas the medicated points, the release of medicament was slow but remained for longer duration of time.

For medicated gutta percha points chlorhexidine impregnated points (Activ points), showed inhibitions zones at 24 hrs and were even more effective at 7 days than $Ca(OH)_2$ points. This may be due to the rapid release of CHX out of the points.

These findings are in agreement with previous studies, (Gomes et al. 2003, Menezes et al. 2003, Siren et al. 2004, Oztan et al. 2006) showed the effectivity of CHX medicament as a possible alternative to calcium hydroxide as intracanal medicament in cases, where *E.faecalis* is suspected.

According to **Moorer** and **Genet**[26] (1983) there is slight antibacterial activity of conventional gutta percha also, presumably due to presence of zinc oxide in the composition. But in the present study conventional gutta percha points did not show any antimicrobial activity because zinc oxide present in it is too weak to be an effective microbiocide.

CONCLUSION

Within the boundaries of this study conventional intracanal medicaments and medicated gutta percha points showed antimicriobial activity against *E. faecalis*, which is main bacteria implicated in post treatment diseases. The medicated gutta percha points showed antibacterial activity for longer duration. But the medicament that is efficient against single bacteria *in vitro* may not be necessarily effective *in vivo*, as

endodontic infections are primarily polymicrobial containing multiple microorganisms. Future research needs to be conducted to evaluate the antimicrobial efficacy of medicated gutta-percha points in clinical conditions against different microbes.

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