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Research Article

To compare the resistance against bacterial micro leakage offered by Zinc Oxide Eugenol sealer, Apexit, AH plus, AH 26 against *Enterococcus Faecalis* along obturated root canals with failed coronal seals

Dr. Rahul Rishi[1], Dr. Gautam Singh[2]
[1]Department of Conservative Dentistry and Endodontics, Passed Darshan Dental College & Hospital, Udaipur, Rajasthan, India
[2]Reader, Department of Conservative Dentistry and Endodontics, Peoples Dental College & Hospital, Bhopal, Madhya Pradesh, India

***Corresponding author**
Dr. Rahul Rishi
Email: rahulrishi2886@gmail.com

Abstract: Coronal micro leakage is one of the reasons for failure of endodontic treatment. Methods to measure coronal micro leakage have included dyes, radioisotopes, fluid filtration, and microorganisms. 52 extracted human lower premolars were previously collected and were stored in saline till it was required for the study, and were divided into 5 groups n=10 .Root canal treatment was done using conventional method and obturation was done using different sealer for each specific group. Group 0 : Control Group (teeth obturated without using a sealer) Group 1:Zinc Oxide Eugenol, Group 2: AH Plus, Group 3: AH 26, Group 4: Apexit. Following this split chamber model was prepared and the samples were labelled and arranged in a test tube stand and incubated at 37^{o}C. Bacterial inoculation was done and any turbidity in the lower chamber was recorded according to the assigned groups for the specific day and staining was done every day. In results the AH 26 (GROUP 3) performed better than AH PLUS (GROUP 2), APEXIT (GROUP 4) and ZOE (GROUP 1). In conclusion AH 26 showed maximum resistance against E. Faecalis.
Keywords: AH 26, *E. Faecalis*resistance, Endodontic sealers, Split Chamber Model.

INTRODUCTION

The basic principle of endodontic practice is to prevent and cure endodontic disease and apical periodontitis when required. To achieve this goal, the endodontic treatment should be based on sound biological rationale, consisting of elimination of the bacteria, their by products and their substrates by disrupting and then destroying the microbial ecosystem through chemical and mechanical methods [1].

Clinicians have strived to totally seal the root canal system in their attempt to ensure endodontic success. Despite these efforts, it has been shown that root canal filling leak [2]. If the coronal portion of the root canal is exposed to the oral environment, the obturated canal acts as potential route for microorganism to gain access into the peri apical environment. This situation may lead to endodontic failure. Missing or fracture restorations, restorations with inadequate margins, recurrent decay, or fractured tooth structure are all clinical conditions that can predispose tooth to coronal micro leakage [2].

Numerous studies have demonstrated the ability of microorganisms and saliva to penetrate an obturated canal and reach the apical region. Swanson and Madison3 Trope et al. demonstrated that endo toxin from Actino bacillus Actinomycetemcomitans was able to pass through obturated root canals within 20 days.

Methods to measure coronal micro leakage have included the use of dyes, radioisotopes fluid filtration, and microorganisms. For each of these methods, the inadequacies have been highlighted and clinical significance questioned [2]. In this study the bacteria chosen was *Enterococcus faecalis* which has been used in various studies done previously and thus more clinically relevant endodontically. In this study various common, clinically used root canal sealers were used and tested for their resistance against *Enterococcus faecalis* along obturated root canal with failed coronal seal.

MATERIALS & METHOD

Preparation of The Teeth Samples

A total of 52 extracted human lower premolars were previously collected and were stored in saline till

it was required for the study. Access opening for all the teeth were performed with a high speed air rotor hand piece (NSK PANA-AIR) with water coolant, the initial entry was made with a 08 round diamond point (MANI). The specimen were instrumented to working length using ISO K file #60 (DENTSPLY MALLIEFER) following step back technique with recapitulation after every instrument used. The canals were copiously irrigated with 5 ml of 3% Sodium Hypochlorite (VISHAL DENTOCARE PRIVATE LIMETED) between each instrument.

The smear layer was removed using EDTA (GLYDETM, DENTSPLY MAILLEFER). The teeth samples were then flushed finally with saline as a final rinse. The roots were kept on a wet gauze to maintain humidity and autoclaved in sterilization pouches sealed with a pouch sealer for 15 mins at 121 ^{o}C at 15 lbs of pressure.

After sterilization, 2 samples were inoculated aseptically in a laminar flow cabinet in BHI broth (HIMEDIA) and incubated anaerobically for 24 hours at 37^{o} C to confirm sterility of the samples. After no growth was found in the broth all the study was performed in aseptic conditions and the teeth were randomly divided into 4 groups with 10 samples in each group.

The root canal sealer used were
Group 0- control
Group 1- Zinc oxide Eugenol (VISHAL DENTOCARE PRIVATE LIMETED, Ahemdabad, India)
Group 2- AH Plus (DENTSPLY Caulk)
Group 3- AH 26 (DENTSPLY Caulk)
Group 4- Apexit (IVOCLAR VIVADENT)

OBTURATION OF THE SAMPLES

Sodium Hypochlorite was used as chemical disinfectant which was efficient for sterilization of 2% Gutta Percha cones (DENTSPLY MAILLEFER) by immersion for only 1 minute. The roots were obturated with master cone of size 60 and the accessory cones with lateral condensation method using gutta percha points and the respective sealer. The excess gutta percha was cut with the help of gutta percha cutting scissors and reduced till the cemento enamel junction with the help of a heated ball burnisher. The samples were allowed to set for 24 hours. The quality of the obturation was evaluated with radiographs by experienced endodontists and the samples with voids were replaced with new samples.

MICROLEAKAGE APPARATUS SET UP

15 ml of sterilized centrifuge tubes were taken and the bottoms cut open for insertion of the samples from within and sealed with epoxy resin to ensure an air tight seal. Then the centrifuge tube was loaded with freshly prepared culture of *Enterococcus faecalis* in BHI broth and the cap was closed tightly over it. This set up was taken and placed over another test tube containing sterilized freshly prepared BHI broth. The samples were labelled and arranged in a test tube stand and incubated at 37^{o}C. Any turbidity in the lower chamber was recorded according to the assigned groups for the specific day and staining was done every day. The broth was changed in the above chamber once in two weeks to maintain an effective number of microbes available for effective micro leakage apparatus.

CULTURING OF THE BACTERIAL STRAIN

20 ml of BHI broth was taken in a test tube and heated on a Bunsen burner for 60 seconds and allowed to cool to reach room temperature. The freeze dried vacuum sealed bacterial sample was opened from one end and the interior sample was placed and mixed in BHI broth by shaking the sample into the broth followed by moving the test tube which was then kept in the incubator for 4 hours before inoculation. Each group was recorded for micro leakage according to the number of days it resisted. The mean for each group was calculated and intergroup comparision was done using one way annova using SPSS software version 19.0

RESULTS

All of the positive controls leaked within 1 day. Throughout the duration of the experiment, group 1, or group 2. In group 3 & group 4 without the coronal barrier displayed turbidity within a range of 6 to 30 days in the present study group 3 showed significant resistances to *E. Faecalis* whereas group 2 & group 4 showed no significant difference and group 1 showed minimum resistance to *E. Faecalis*.

Table-1: Mean no of days in which leakage occurred

Descriptive						
Days					95% Confidence Interval for Mean	
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
0(control)	10	1.00	.000	.000	1.00	1.00
1(ZOE)	10	8.90	2.846	.900	6.86	10.94
2(AHPLUS)	10	16.60	2.591	.819	14.75	18.45
3(AH26)	10	24.00	2.749	.869	22.03	25.97
4(APEXIT)	10	21.90	2.558	.809	20.07	23.73
Total	50	14.48	8.911	1.260	11.95	17.01

Table- 2: Descriptive

Days		
	Minimum	Maximum
0	1	1
1	6	15
2	14	22
3	21	30
4	20	27
Total	1	30

Table-3: Inter Group Comparison

ANOVA					
Days					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3630.280	4	907.570	156.959	.000
Within Groups	260.200	45	5.782		
Total	3890.480	49			

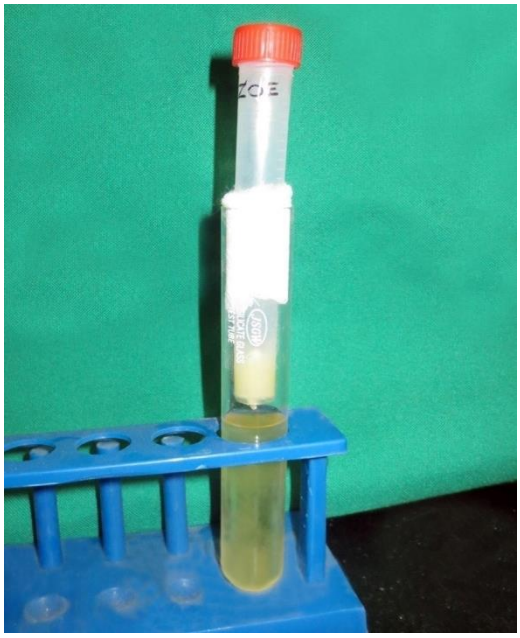

Fig- 1: split chamber model

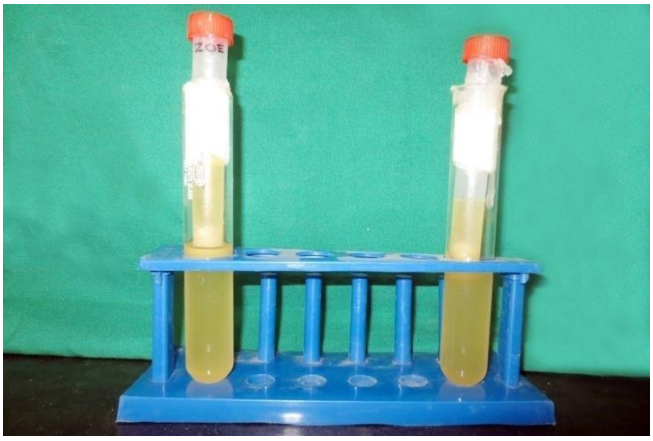

Fig-2: Turbidity seen after bacterial invasion.

DISCUSSION

Various studies demonstrated that in case of coronal leakage microbe invariably pass through obturated root canal over a short period of time [2,3,9]. Coronal micro leakage can occur because of a variety of reasons including premature loss of temporary restoration or inadequate final restoration, Missing or fractured restorations, restorations with inadequate margins, recurrent decay, or fractured tooth structure.

In in-virto studies, *Enterococcus faecalis* has been shown to invade dentinal tubules. It can colonise root canal and survive without the support of other bacteria. It is resistant to the antimicrobial effects of calcium hydroxide, probably partly due to an effective proton pump mechanisms which maintains optimal cytoplasmic pH levels. The rapid emergence of antimicrobial resistance among Enterococci helps to shift the microbial flora in favour of *E. Faecalis*[11]. In our study *Enterococcus faecalis* used as a biological marker which makes the study more clinically significant.

Zinc-Oxide-Eugenol(ZOE) based sealers, such as Roth 811(Roth International Ltd, Chicago, IL) and Kerr EWT(Kerr Corporation , Orange , CA, USA), are known for their antibacterial effect through the action of eugenol, AH –PLUS (DENTSPLY International, York, PA, U.S.A.), which was modified from AH26 (DENTSPLY Caulk), is popular for its tissue compatibility property. AH-PLUS (DENTSPLY Caulk) does not release formaldehyde compared with its predecessor, AH26 [12].

This study evaluated the resistance offered by various commonly used root canal sealers along

obturated root canals in which coronal seal had failed due to causes unknown. The experimental design was similar to previously done study by Barthel et al where "split chamber model" were used for a micro leakage study [4].

Laboratory experiments to measure radicular dentin pH have suggested an inadequate rise in pH in dentinal tubules for effective results. The limited antibacterial activity of calcium hydroxide sealer might be attributed to a lack of sufficient pH elevation, limited solubility, and diffusibility of calcium hydroxide into dentinal tubules and possibly buffering ions present in the tubules [13].

Seal apex releases calcium hydroxide and it showed only slight toxicity in the fresh state. However, it exhibits increasing cytotoxicity in the set state. This might be the reason why Seal apex could exhibit the antimicrobial effect. Resin-based sealers AH26 and AH plus are similar materials, both of them were shown to be antimicrobial [14].

Pizzo *et al.;* reported that in Direct Contact Test only fresh AH Plus possessed antibacterial activity, whereas 24-hour and 7-day-old samples did not show antibacterial effect against *E. Faecalis*, Similar results were reported by Kayaoglu et al . The antimicrobial effect of epoxy resin–based sealers might be related to the release of formaldehyde during the polymerization process. The present study also showed that fresh AH plus had significant antibacterial effect, whereas set samples did not show antimicrobial activity [15].

The antimicrobial effect of resin-based sealers may be related to bisphenol. A diglycidyl ether which was previously identified as a mutagenic component of the resin based material .In addition, both of them have been reported to release formaldehyde in the polymerization process. Taken together, these components made the resin-based sealers antimicrobial [14]. ZOE showed antibacterial activity against K. rhizophila and E. coli, and the smallest zones of bacterial growth inhibition against E. faecal are. Similar results have been reported [16].

Savioli *et al.;* evaluated the antimicrobial activity of a ZOE-based root canal filling material for permanent teeth (Grossman's sealer) and its components against different microbial strains using the double layer well-diffusion method, and found that the eugenol component inhibited K. rhizophila, *E. Faecalis*, S. mutans, E. coli and S. aureus. Zinc Oxide alone inhibited only the growth of S. sobrinus and E. coli. Several authors have attributed the antimicrobial effects of ZOE to eugenol [16].

Although the antibacterial mechanism of ZnO nanoparticles is still unknown, the possibilities of membrane damage caused by direct or electrostatic interaction between ZnO and cell surfaces, cellular internalization of ZnO nanoparticles, and the production of active oxygen species such as H_2O_2 in cells due to metal oxides have been proposed in earlier studies. The generation of H_2O_2 in ZnO slurries was determined by oxygen electrode analysis and spectro photo fluorometry [17].

The AH 26 (GROUP 3) performed better than AH PLUS (GROUP 2), APEXIT (GROUP 4) & ZOE (GROUP 1). This may be due to AH 26 when mixed, the hardener in this material, hexamethylenetetramine, and releases formaldehyde in an amount increasing over the 2-day setting period. Once set, the formaldehyde concentration in ~×200 that of the fresh mix and subsequently decreases over the next 7 days. This condition may be related to antibacterial effects in this sealer[18]. AH PLUS is a modified formulation of AH26 in which formaldehyde is not released[19].

The antimicrobial activity of AH PLUS and APEXIT appear comparable may be because Calcium hydroxide-based sealer was shown to be appropriate for elimination of bacteria. It depends on ionization that releases OH– ions, causing an increase in pH. A pH >9 may reversibly or irreversibly inactivate cellular membrane enzymes of the microorganism, resulting in a loss of biological activity[14]. ZOE (GROUP 1) showed least resistance to *E. Faecalis*. May be because it showed the lowest bond strength to dentin in absence of smear layer, when compared to the resin based sealers[20].

CONCLUSION

Taken together, these findings suggest that the sealers evaluated in this study showed different inhibitory effects on the bacterial strains. Root canal sealers containing formaldehyde proved to be the most effective against the *E. Faecalis* because the antimicrobial components of root canal sealers do not have selective toxicity against microorganisms, they usually exert toxic effects on host cells. We suggest that those root canal sealers should be used which are characterized by an at least acceptable biocompatibility.

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REFERENCES

1. Markus Haapasalo, Trude Udnæs, Unni Endal; Persistent, recurrent, and acquired infection of the

root canal system post-treatment. Endodontic Topics.2003; 6: 29–56.
2. Gary A. Wolanek, Robert J. Loushine, R. Norman Weller, W. Frank Kimbrough, Keith R. Volkmann; In Vitro Bacterial Penetration of Endodontically Treated Teeth Coronally Sealed with a Dentin Bonding Agent. J Endodon. 2001; 27(5): 354-57.
3. Kimberly Swanson, Sandra Madison; An evaluation of coronal micro leakage in endodontically treated teeth. Part I. Time periods. J Endodon. 1987; 13: 56-59.
4. Barthel CR, Zimmer S, West G; Bacterial leakage in obturated root canals following the use of different intracanal medicaments. Endod Dent Traumatol. 2000; 16: 282–86.
5. Trope M, Chow E, Nissan R; In vitro endotoxin penetration of coronally unsealed endodontically treated teeth. Endod Dent Traumatol. 1995; 11: 90-94.
6. Scott W. Edgar, J. Gordon Marshall J. Craig Baumgartner; The Antimicrobial Effect of Chloroform on *Enterococcus faecalis* After Gutta-Percha Removal. J Endodon. 2006; 32 : 1185-1187
7. Augsburger R, Peters D; Radiographic evaluation of extruded obturation materials. J Endod.1990; 10: 492-97.
8. Gutmann JL, Saunders WP, Saunders EM, Nguyen L; An assessment of the plastic Thermafil obturation technique Part 1 Radiographic evaluation of adaptation and placement. Int Endod J.1993; 26:173-178.
9. Khayat A, Lee S, Torabinejad M; Human Saliva penetaration of coronally unsealed obturated root canals. J Endod.1993; 19: 458-61.
10. Nan-Shim Pang, Il-Young Jung, Kwang-Shik Bae, Seung-Ho Baek, Woo-Cheol Lee, *et al.;* Effects of Short-term Chemical Disinfection of Gutta-Percha Cones: Identification of Affected Microbes and Alterations in Surface Texture and Physical Properties. J Endodon.2007; 33: 594-598.
11. Isabelle Portenier, Tuomos MT Waltimo & Markus Haapasalo; *Enterococcus faecalis* – the root canal survivor and 'star' in post treatment disease. Endodontic Topics. 2003; 6: 135–159.
12. Markus Haapasalo, Wei Qian, Isabelle Portenier, Tuomas Waltimo; Effects of Dentin on the Antimicrobial Properties of Endodontic Medicaments. J Endodon. 2007; 33: 917-25.
13. Shalin Desai, Nicholas Chandler; Calcium Hydroxide–Based Root Canal Sealers: A Review. J Endodon. 2009; (35): 475-80.
14. Chung-Chih Lai, Fu-Mei Huang, Hui-Wen Yang, You Chan, Min-Sheng Huang, *et al.;* Antimicrobial activity of four root canal sealers against endodontic pathogens. Clin Oral Invest. 2001; 5: 236–39.
15. Hui Zhang, Ya Shen, N. Dorin Ruse, Markus Haapasalo; Antibacterial Activity of Endodontic Sealers by Modified Direct Contact Test Against *Enterococcus faecalis*. J Endodon. 2009; 35: 1051-55.
16. Alexandra Mussolino de Queiroz, Paulo Nelson Filho Léa Assed Bezerra da Silva, Sada Assed, Raquel Assed Bezerra da Silva, *et al.;* Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Seal apex and EndoREZ. Braz. Dent. J. 2009; 20: 290-96.
17. Yanping Xie, Yiping He, Peter L. Irwin, Tony Jin, Xianming Shi; Antibacterial Activity and Mechanism of Action of Zinc Oxide Nanoparticles against Campylobacter jejuni. Appl. Environ. Microbiol. 2011; 77: 2325-31.
18. Hasan Razmi1, Kazem Ashofteh Yazdi1, Fereshteh Jabalameli, Shaghayegh Parvizi; Antimicrobial effects of AH26 sealer/antibiotic combinations against *Enterococcus faecalis*. Int Endod J .2008; 3: 107-112.
19. Kenneth M. Hargreaves, Stephen Cohen. Cohen's pathways of the pulp.10th ed. New Delhi: Elsevier; 2011.
20. João Vicente Baroni Barbizam, Martin Trope, Mario Tanomaru-Filho, Erica Cappelletto Nogueira Teixeira, Fabricio Batista Teixeira; Bond strength of different endodontic sealers to dentin: push-out test. J Appl Oral Sci. 2011; 19: 644 - 47.