

Evaluation of Antibacterial Efficacy of MTA with and without Additives Like Silver Zeolite and Chlorhexidine

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ABSTRACT

Introduction: Microorganisms, predominantly *Enterococcus faecalis* are found responsible in the progression of pulpal, periradicular diseases and in endodontic failures. Unsuccessful conventional treatment might necessitate the need for a surgical approach; where in retrograde restorative materials are used to seal the apex. Among the root end filling materials, Mineral Trioxide Aggregate (MTA) is considered biocompatible and is most commonly used in clinical applications but it has limited antibacterial activity. Metallic silver and chlorhexidine have been added into various dental materials to enhance the antibacterial activity.

Aim: This study aimed to compare the antibacterial effect of MTA, MTA mixed with silver zeolite and MTA mixed with chlorhexidine against *Enterococcus faecalis*.

Materials and Methods: Test materials used in the study were divided into three groups namely Group 1- MTA, Group 2-MTA + Silver Zeolite, Group 3-MTA + Chlorhexidine. Direct contact test was done by placing a standardized suspension of *Enterococcus faecalis* on the test materials in a 96 well microtiter plate. The bacterial growth was measured spectrophotometrically using ELISA reader at intervals of one, three and seven days.

Statistical Analysis: Data was collected by recording the optical density and analyzed using two-way ANOVA and Tukey's multiple post hoc test followed by paired-t test.

Results and Conclusion: All test groups showed antibacterial activity against *Enterococcus faecalis* at day one, three and seven. MTA with silver zeolite showed the maximum antibacterial activity followed by MTA with 2% chlorhexidine. The least antibacterial effect was shown by MTA mixed with sterile water.

Keywords: Antibacterial additives, Direct contact test, *Enterococcus faecalis*, Root end filling materials

INTRODUCTION

The endodontic treatment aims at cleaning, shaping followed by complete obturation of the root canal system [1]. Failure after endodontic therapy is due to persistent or secondary infection especially of *Enterococcus faecalis* in poorly and in well treated root canals [2]. Surgical intervention aims to remove the infected root end and ensure good root canal sealing using root end filling materials. Among the numerous root end filling materials available MTA is most frequently used and has shown long term clinical success [3]. However some studies showed MTA to have no effect or to delay the growth of *Enterococcus faecalis* [4-6].

Incorporation of certain additives like metallic silver and chlorhexidine has shown to enhance antibacterial properties of various dental materials [7,8]. So the purpose of the study was to assess whether incorporation of these additives enhance the antibacterial efficacy of MTA especially against *E. Faecalis*.

Antibacterial efficacy of root end filling materials can be evaluated using agar diffusion test, however it was found to be insensitive and unable to differentiate with bactericidal or bacteriostatic ability [9]. Therefore a more appropriate, quantitative and reproducible test such as direct contact test would be more appropriate [10].

AIM

So the present study aimed to evaluate the antibacterial effect of MTA, MTA mixed with silver zeolite and MTA mixed with chlorhexidine against *Enterococcus faecalis* using direct contact test.

MATERIALS AND METHODS

This in-vitro study was conducted in Department of Conservative Dentistry and Endodontics in collaboration with Department of Microbiology, KLE's VK Institute of Dental Sciences, Belgaum, Karnataka, India in the year 2013.

The study had three main groups based on the addition of antibacterial agents to MTA as follows:

Group 1: Mineral Trioxide Aggregate (ProRoot MTA, Dentsply) was dispensed and mixed with sterile distilled water in the ratio of 1:1 as the control group.

Group 2: Powder form of silver zeolite (Sigma Aldrich Chemie GmbH, Germany, Batch #06306CJ.) was added at 2% mass fraction to Mineral Trioxide Aggregate powder and mixed with sterile distilled water.

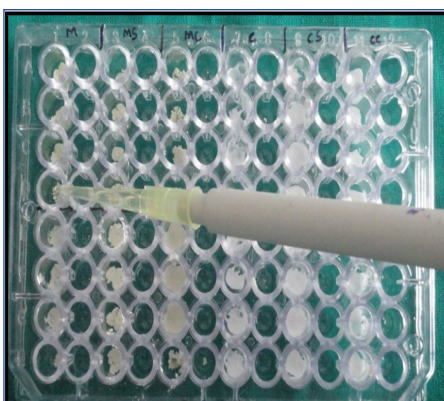
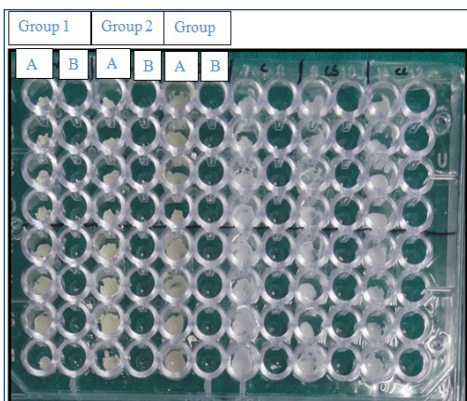
Group 3: Mineral Trioxide Aggregate was mixed with 2% chlorhexidine (V – Consept) in 1:1 ratio.

Test Microorganism

Enterococcus faecalis (ATCC 29212) was grown on brain heart infusion agar plate and then sub-cultured on nutrient agar medium. After confirming the purity of the strain, the bacterial suspension was inoculated into 5ml of 85% saline and adjusted spectrophotometrically at 800nm to match the transmittance of 90T (equivalent to 0.5 McFarland scale = 1.5×10^8 C.F.U) [11].

Direct Contact Test [10]

Direct contact test is based on determining the turbidity of bacterial growth in 96 well microtiter plates. In the present study all the test materials were freshly mixed and were placed at the bottom of four wells in the microtiter plate (subgroup A) to a height of 2mm which were then exposed to 10 μ l bacterial suspension [Table/Fig-1]. BHI broth (245 μ l) was added after ensuring direct contact of the bacteria to the test material [Table/Fig-2]. After mixing for 2 min, 15 μ l was transferred into four adjacent wells (subgroup B) containing 215 μ l fresh medium. The kinetics of bacterial outgrowth in each well were then followed by continuous measurements by ELISA reader measured at 630 nm [Table/Fig-3]. Densitometric readings were taken on the first, third and seventh day from each set of samples. The experiments were triplicated.



[Table/Fig-1]: Wells coated with test materials. **[Table/Fig-2]:** Transfer of brain heart infusion broth to the microtiter wells. **[Table/Fig-3]:** Elisa Reader (Stat Fax 2100) (Awareness Technology).

SV	DF	SS	MSS	F-value	p-value
Main effects					
Groups	2	0.0431	0.0215	485.1430	<0.0001
Sub groups	1	0.0124	0.0124	279.3269	<0.0001
2-way interactions					
Groups x Sub groups	2	0.0020	0.0010	22.4702	<0.0001
Error	18	0.0008	0.0000		
Total	23	0.0583			

[Table/Fig-4]: Two way ANOVA between main groups (1,2 and 3) and sub groups (A and B) with respect to optical density at 1st day.
*Abbreviations: SV-Sources of variation, DF-Degrees of freedom, SS-Sum of squares, MSS-Mean sum of squares, p-Probability, SD-Standard deviation

Main groups		Group 1		Group 2		Group 3	
Sub groups		Sub group A	Sub group B	Sub group A	Sub group B	Sub group A	Sub group B
Mean		0.2649	0.1940	0.1402	0.1112	0.1931	0.1567
SD		0.0112	0.0035	0.0021	0.0063	0.0067	0.0063
Group 1	Sub group 1	-					
	Sub group 2	0.0002*	-				
Group 2	Sub group 1	0.0002*	0.0002*	-			
	Sub group 2	0.0002*	0.0002*	0.0002*	-		
Group 3	Sub group 1	0.0002*	0.9998	0.0002*	0.0002*	-	
	Sub group 2	0.0002*	0.0002*	0.0271*	0.0002*	0.0002*	-

[Table/Fig-5]: Pair wise comparison of main groups (1, 2 and 3) and sub groups (A and B) with respect to optical density at 1st day by Tukey's multiple post hoc procedures.
*p<0.05

SV	DF	SS	MSS	F-value	p-value
Main effects					
Groups	2	0.0453	0.0226	408.4914	<0.0001
Sub groups	1	0.0172	0.0172	309.4468	<0.0001
2-way interactions					
Groups x Sub groups	2	0.0006	0.0003	5.5780	0.0130*
Error	18	0.0010	0.0001		
Total	23	0.0641			

[Table/Fig-6]: Two way ANOVA between main groups (1,2 and 3) and sub groups (A and B) with respect to optical density at 3rd day.
*Abbreviations: SV-Sources of variation, DF-Degrees of freedom, SS-Sum of squares, MSS-Mean sum of squares, p-Probability, SD-Standard deviation

Main groups		Group 1		Group 2		Group 3	
Sub groups		Sub group A	Sub group B	Sub group A	Sub group B	Sub group A	Sub group B
Mean		0.3455	0.2954	0.2367	0.1936	0.2883	0.2211
SD		0.0066	0.0085	0.0111	0.0033	0.0070	0.0059
Group 1	Sub group 1	-					
	Sub group 2	0.0002*	-				
Group 2	Sub group 1	0.0002*	0.0002*	-			
	Sub group 2	0.0002*	0.0002*	0.0002*	-		
Group 3	Sub group 1	0.0002*	0.7571	0.0002*	0.0002*	-	
	Sub group 2	0.0002*	0.0002*	0.0765	0.0008*	0.0002*	-

[Table/Fig-7]: Pair wise comparison of main groups (1, 2 and 3) and sub groups (A and B) with respect to optical density at 3rd day by Tukey's multiple post hoc procedures.
*p<0.05

STATISTICAL ANALYSIS

All the data collected by recording the optical density were analyzed using two-way ANOVA and Tukey's multiple post hoc test followed by paired-t test.

RESULTS

According to the results of the study, two way ANOVA and Tukey's multiple post hoc test showed that MTA mixed with silver zeolite (Group 2) had maximum antibacterial activity followed by MTA mixed with 2% chlorhexidine (Group 3). The least antibacterial activity was seen with MTA mixed with sterile distilled water (Group 1) in fresh as well as in aged samples of three and seven days. The optical densities at day one were 0.2649±0.0112, 0.1402±0.0021 and 0.1931±0.0067 for Group 1, 2, 3 respectively [Table/Fig-4, 5]. At day three, optical densities were 0.3455±0.0066, 0.2367±0.0111, 0.2883±0.0070 [Table/Fig-6,7] and for day seven they were

0.4598±0.0014, 0.3411±0.0039, 0.3924±0.0099 for Group 1, 2 and 3 respectively [Table/Fig-8,9]. There was significant difference (p<0.05) in the optical density among the three test materials at all time intervals.

DISCUSSION

Complete elimination of the bacteria from the pulp space plays an important role in the success of endodontic treatment. Post-endodontic failures are due to inadequate cleaning and shaping and/or insufficient obturation. Microorganisms have been isolated from 35% to 100% of failed cases, predominantly *E. faecalis* [11]. Hence *E. faecalis* was taken as test organism in the study.

Failure after conventional endodontic treatment necessitates surgical intervention which aims to remove the infected root end and ensure good sealing by root end filling materials, among which MTA seems to be promising; however its antibacterial properties

SV	DF	SS	MSS	F-value	p-value
Main effects					
Groups	2	0.0531	0.0266	245.3901	<0.0001
Sub groups	1	0.0246	0.0246	226.8104	<0.0001
2-way interactions					
Groups x Sub groups	2	0.0001	0.0001	0.5199	0.6033
Error	18	0.0019	0.0001		
Total	23	0.0797			

[Table/Fig-8]: Two way ANOVA between main groups (1,2 and 3) and sub groups (A and B) with respect to optical density at 7th day.
*Abbreviations: SV-Sources of variation, DF-Degrees of freedom, SS-Sum of squares, MSS-Mean sum of squares, p-Probability, SD-Standard deviation

Main groups		Group 1		Group 2		Group 3	
Sub groups		Sub group A	Sub group B	Sub group A	Sub group B	Sub group A	Sub group B
Mean		0.4598	0.3900	0.3411	0.2785	0.3924	0.3329
SD		0.0114	0.0096	0.0039	0.0032	0.0099	0.0175
Group 1	Sub group 1	-					
	Sub group 2	0.0002*	-				
Group 2	Sub group 1	0.0002*	0.0002*	-			
	Sub group 2	0.0002*	0.0002*	0.0002*	-		
Group 3	Sub group 1	0.0002*	0.9994	0.0002*	0.0002*	-	
	Sub group 2	0.0002*	0.0002*	0.8710	0.0002*	0.0002*	-

[Table/Fig-9]: Pair wise comparison of main groups (1, 2 and 3) and sub groups (A and B) with respect to optical density at 7th day by Tukey's multiple post hoc procedures.
*p<0.05

are controversial [12]. Grey MTA was found to invoke a more desirable biologic response than White MTA and hence should be used as root end filling material as esthetics is not the prime concern at these sites [13]. Hence in the study ProRoot MTA was used.

Addition of silver containing zeolite to materials like GIC and resins has improved their antimicrobial activity [7,14]. Chlorhexidine (CHX) is a known antimicrobial agent especially against *E. Faecalis* along with biocompatibility with periodontal tissues [15]. Luddin N, and Ahmed HM have showed CHX to be effective in reducing or completely eliminating *E. faecalis* [15,16]. Hence in this study silver zeolite and chlorhexidine were used as additives with MTA to increase its antibacterial property.

Antibacterial activity has been evaluated using agar diffusion test in in-vitro studies but it has shown to lack inoculum standardization, culture adequacy, sample size etc.,[17]. To overcome these disadvantages, a standardized protocol suggested by Weiss et al., that is direct contact test was followed [10]. Direct contact test is a qualitative and reproducible method, which is ineffective and insensitive to the inoculum size brought in contact to the test material.

According to the results of this study, antimicrobial activity of MTA mixed with silver zeolite was maximum followed by MTA mixed with chlorhexidine. MTA mixed with sterile water showed least antimicrobial activity at all the time intervals tested. These results are similar to findings of Eldeniz and Hasan Zarrabi M et al., whose study showed that MTA was ineffective against *E. faecalis* [6,18]. In contrast, Al-Hezaimi K et al., stated that gray MTA was effective against *E. faecalis* [19]. The ineffectiveness of MTA on *E. faecalis* in most of studies is due to the capacity of this microorganism to survive under various stressful environmental condition [20]. This

could be the reason for low antimicrobial activity of MTA against *E. Faecalis*.

Silver zeolite incorporation enhanced the antimicrobial property of MTA, which was also confirmed by Odabas ME et al., [21]. Silver zeolite shows antimicrobial activity because of metallic silver as its constituent, as it has highest antibacterial activity among metal ions. Zeolite is a porous crystalline material of hydrated sodium and aluminosilicate, having void spaces within frameworks of 3-10 angstroms in diameter that are capable of hosting cations such as silver or zinc [22]. Kawahara suggested silver zeolite as a useful vehicle to enhance the antibacterial activity of the dental materials [23]. Davies showed that antimicrobial activity of glass ionomer cement could be enhanced by incorporating silver zeolite with it [24].

MTA with 2% chlorhexidine showed good antibacterial activity as compared to MTA alone; however it was less effective as compared with MTA and silver zeolite. This could be attributed to the chlorhexidine crossing the microbial cell wall by passive diffusion, and disturbing the cytoplasmic membrane causing reduction in leakage, leading to lysis. Similar results were shown by Stowe who substituted chlorhexidine gluconate for water to enhance the antimicrobial activity of ProRoot MTA [25].

LIMITATION

To fully access the viability of silver zeolite MTA, further studies are needed to evaluate the physical properties and test antibacterial effect in clinical conditions.

CONCLUSION

Under the parameters of the study, it can be concluded that all the test materials exhibited antibacterial activity against *E. faecalis*. MTA mixed with silver zeolite showed the maximum antibacterial activity followed by MTA mixed with 2% chlorhexidine and the least shown by MTA mixed with sterile water. Silver zeolite and chlorhexidine can be incorporated into MTA to enhance its antibacterial effect against *E. Faecalis*.

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