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Original Research

Efficacy of MTA, MTA Plus, Biodentine on E. faecalis biofilm

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ABSTRACT:
Background: Enterococcus faecalis are Gram-positive cocci, facultative anaerobes and are commonly detected in asymptomatic, persistent endodontic infections. The present study compared efficacy of MTA, MTA Plus, Biodentine on E. faecalis biofilm. **Materials & Methods:** The present invitro study comprised of MTA, MTA Plus, Biodentine, and Chitosan. Gram-positive bacterium tested-E. faecalis ATCC 29212 was used in the study. **Results:** Group I had MTA, group II had MTA Plus, and group III had Biodentine. The mean optical density in group I was 0.18, in group II was 0.23 and in group III was 0.29. The difference was non- significant (P> 0.05). **Conclusion:** All the materials proved to have antibiofilm action against E. faecalis.
Key words: antibiofilm, optical density, E. faecalis.

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INTRODUCTION

Biofilms are sessile microbial communities composed of cells irreversibly attached to a substratum and interface or to each other.[1] Ultrastructurally biofilms form tower- or mushroom-shaped microcolonies with interspersed channels that are separate from the external environment and through which fluids move by convection.[2,3] The cells within biofilms produce the matrix of extracellular polymeric substance. Cells located more deeply in the biofilm are exposed to environmental conditions that differ from those at the surface including decreased oxygen tension.[4] This results in altered phenotypes in terms of growth rate and gene transcription that might facilitate certain survival and virulence characteristics. The slow metabolic rate of microorganisms in biofilms as well as the extracellular matrix of the biofilm can impede the effectiveness of many antimicrobials.[5]

Enterococcus faecalis are Gram-positive cocci, facultative anaerobes and are commonly detected in asymptomatic, persistent endodontic infections. Its prevalence in such infections ranges from 24% to 77%. Studies have established the ability of E. faecalis to resist starvation and develop biofilms under nutrient-deprived conditions.[6] Biofilms formed by E. faecalis are able to resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing bacteria. Root canal failures are likely to occur due to the persistence of bacteria especially E. faecalis and their by-products in root canal system.[7] This bacterium appears to be highly resistant to the anti-bacterial effect of Ca $(OH)_2$. The present study compared efficacy of MTA, MTA Plus, Biodentine on E. faecalis biofilm.

MATERIALS & METHODS

The present invitro study comprised of MTA, MTA Plus, Biodentine, and Chitosan. Gram-positive

bacterium tested-E. faecalis ATCC 29212 was used in the study.

A 3-day biofilm was generated in a 96 well microtiter plate. Biofilm was grown at 37°C (2 ml Brain Heart Infusion Broth [BHI] containing 0.5% sucrose), and media was changed every 24 h. At the end of the 3rd day, each disc was rinsed with phosphate-buffered saline (PBS) to remove loosely attached bacteria and planktonic bacteria. 200 µl of overnight trypticase soy broth culture was added to wells and incubated at 37°C for 24 hours. Group I had MTA, group II had MTA Plus, and group III had Biodentine. A serial two-fold dilutions of the combinations were prepared in PBS and incubated for 2 hours at room temperature to check for minimal inhibitory concentration (MIC) of the materials.

After 3 days of incubation, wells were aspirated and 100 µl of material and their conjugates were added and incubated at 37°C for 24 hours. Each sample was added in triplicate and un-inoculated broth was used as control. Optical density of adherent stained biofilm was read at 590 nm using ELISA auto reader. Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

RESULTS

Table I Distribution of material

Groups	Group I	Group II	Group III
Materials	MTA	MTA plus	Biodentine

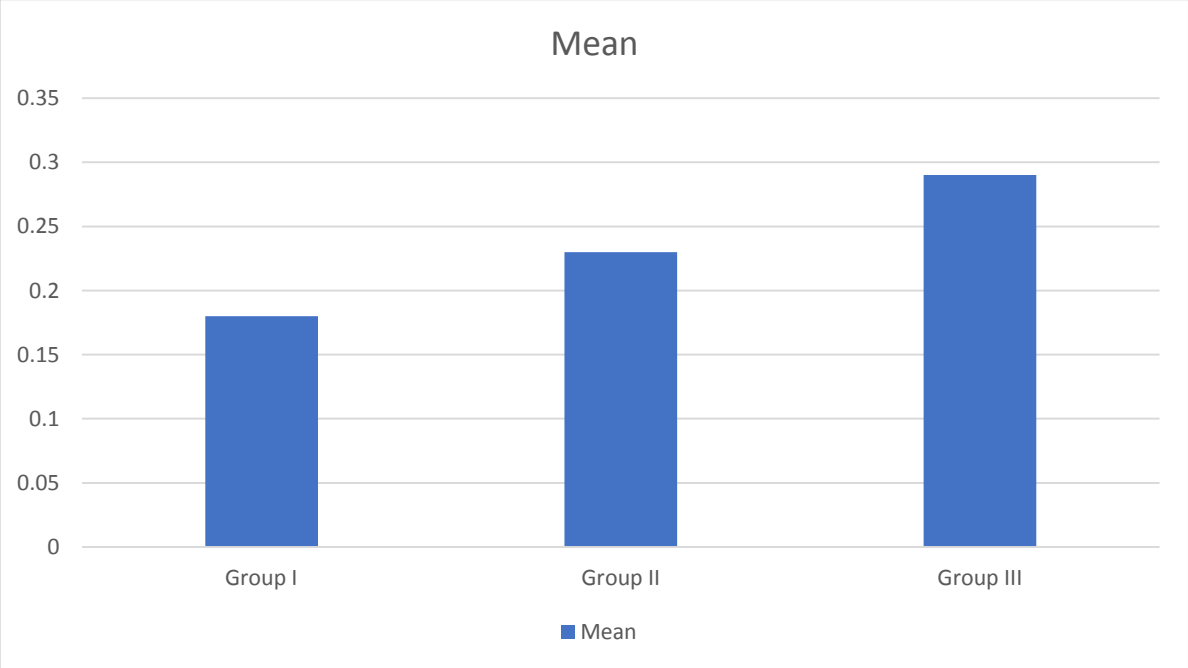
Table I shows distribution of materials in various groups. Group I had MTA, group II had MTA Plus, and group III had Biodentine.

Table II Comparison of optical density

Groups	Mean	P value
Group I	0.18	0.61
Group II	0.23	
Group III	0.29	

Table II, graph II shows that mean optical density in group I was 0.18, in group II was 0.23 and in group III was 0.29. The difference was non- significant (P> 0.05).

Graph II Comparison of optical density



DISCUSSION

Biofilm is a complex structure adhering to surfaces that are regularly in contact with water, consisting of colonies of bacteria and usually other microorganisms such as yeasts, fungi, and protozoa that secrete a mucilaginous protective coating in which they are encased.[8,9] Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other are embedded in a matrix of extra poly saccharide (EPS) that they have produced and exhibit an altered phenotype with respect to growth rate and gene transcription.[10] Endodontic biofilm is established to be less diverse compared to the oral biofilm. The progression of infection alters the nutritional and environmental status within the root canal.[11] The root

canal environment apparently becomes more anaerobic and the nutritional level will be depleted. These changes will offer a tough ecological niche for the surviving microorganisms. Intracanal microbial biofilms are formed on the root canal dentine of an endodontically infected tooth.[12] The present study compared efficacy of MTA, MTA Plus, Biodentine on E. faecalis biofilm.

In present study, mean optical density in group I was 0.18, in group II was 0.23 and in group III was 0.29. Hiremath et al[13] evaluated the antibiofilm activity of root end materials against Enterococcus faecalis. Mineral trioxide aggregate (MTA), MTA plus and Biodentine were conjugated with chitosan gel and tested against the 3-day biofilm of E. faecalis. The incubated plates were stained using crystal violet stain and the optical density of adherent stained biofilm was read at 590 nm using ELISA auto reader. There was a mean clinical reduction in the biofilms of the conjugates as compared to their individual counter parts. There was a statistically significant difference seen between the groups (MTA Plus – Chitosan Conjugate) and (MTA – Chitosan Conjugate) with $P = 0.0495$. Duggan et al[14] tested the hypothesis that the ability of Enterococcus faecalis to form biofilms is related to the source of the strains. E. faecalis strains recovered from root canals, the oral cavity and non-oral/non-endodontic sources were studied. Biofilms were grown in tryptic soy broth in 96-well plates for 24 hours at 37°C, fixed with Bouin's fixative, and stained with 1% crystal violet. Optical density at 570 nm (OD570) was measured by using a microtiter plate reader. Experiments were performed in quadruplicate on three occasions and mean OD570 readings determined for each strain. There were no statistically significant differences between groups. Within the root canal and oral isolates there were no significant associations between biofilm formation and the presence of the virulence determinants asa, cylA, esp, and gelE.

CONCLUSION

Authors found that all the materials proved to have antibiofilm action against E. faecalis.

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