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ANTIBACTERIAL EFFICIENCY OF MINERAL TRI OXIDE AGGREGATE AND BIODENTIN IMPREGNATED WITH SILVER AND ZINC OXIDE NANOPARTICLES- AN *INVITRO* STUDY

Dental Science

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ABSTRACT

Aim: The aim of this in-vitro study is to compare the antibacterial efficacy of Mineral trioxide aggregate (MTA) and Biodentin along with the incorporation of silver and Zinc oxide Nano particles.

Materials and methodology: Antimicrobial effect of MTA and Biodentin impregnated with silver and Zinc oxide Nano particles were tested against Enterococcus faecalis, Lactobacillus and Candida albicans. The minimal inhibitory concentration of the following materials was measured and the antimicrobial effect was determined bothquantitatively and qualitatively by using Brothdilution method and membrane enclosed immersion test . The data's were statistically analyzed using SPSS 21 IBM software and comparison between the two groups in normal data distribution were done using Student's t-test.

Results: Test indicates that the antimicrobial activity of Biodentine with silver nano particles showed very strong antibacterial action compared with other materials to the three bacterial strains followed by MTA and Silver Nano particles. There was a statistically significant difference between these materials

Conclusion: By quantifying the amount of nanoparticles that can be added to the bioactive materials These can be successfully used in the clinical practice.

KEYWORDS

Biodentin, MTA, Nanoparticles, Minimal inhibitory concentration.

INTRODUCTION

The remarkable evolution in the field of dentistry is the invention of numerous bioactive materials with good sealing ability and biocompatibility. In spite of their excellent properties, the major problem that come across is the microscopic space that are created across the preparation and material interface which paves the way for the bacteria and their byproducts to penetrate and harbor at the particular place causing reinfection. [1] So along with the good sealing ability this materials should also possess antimicrobial effect for better clinical outcome.[2]

Mineral trioxide aggregate, a commonly used hydrophilic cement contains 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight along with trace amounts of sulphur, magnesium, phosphorus, sodium sulphate and potassium sulphate. The fine hydrophilic particles in the presence of water form a colloidal gel which on solidification forms hard cement within approximately 4 hours. [3] It has wide range of endodontic applications such as root end filling ,pulp capping, apexification, root wall perforation and resorptions etc.[4]

Another newer endodontic bioactive material is the Biodentine (Septodont) in which the powder mainly consists of tricalcium and dicalcium silicate and calcium carbonate. The liquid contains aqueous solution of calcium chloride with a polycarboxylate admixture, which sets in 12 min.[5]

Silver, for centuries has been used in medicine because of its excellent antimicrobial properties. More recently, silver in Nano particulate form have been synthesized and incorporated into several commonly used biomaterials in which their small size provides great antimicrobial effect. So these nanoparticles have been recently applied in dentistry to improve the antibacterial effect thus reducing the biofilm formation over dental materials surfaces.[6]

Zinc oxide powders also possess antimicrobial properties several other that have been specifically shown to kill several oral microbes that are known to contribute to caries. Nanoparticles of ZnO (NPs) have been found to be more effective than larger particles against both gram negative and gram positive bacteria.(7)

Nanotechnology provides a wide range of possibilities to develop newer antimicrobial materials .(8)However, the disadvantage that was reported is the color change which is an important property of dental materials .In this study, the antimicrobial activity of MTA and Biodentine was evaluated when these materials are mixed with silver and zinc oxide nano particles .

MATERIAL AND METHODS

Microbial culture

In this study, three microorganism species namely Enterococcus Faecalis(MTCC 439), Lactobacillus and Candida albicans were selected against the dental bioactive materials. The cultures were grown and maintained in Brain Heart Infusion broth (BHI, Difco, Detroit, MI, USA) and was supplemented with 10% heat-inactivated blood serum to improve its growth. The culture of the three bacterial strains was incubated for 16h at 37°C under anaerobic conditions.

Material preparation

MTA Angelus was manipulated using a sterile glass plate and spatula in the ratio of 1 g powder to 0.33 g sterile distilled water, according to the manufacturer's instructions. Biodentine is prepared by adding 5 drops of liquid to the powder. These components are then triturated in an amalgamator for 30 s at 4000 rpm which leads to the formation of a paste of creamy consistency of the cement.The silver and zinc oxide nanoparticles and mixed with those materials in 1% weight.

Determination of MIC

The antimicrobial efficacy of MTA, Biodentine, MTA+Silver Nano particles, Biodentine+silver nanoparticles (Ag-NPs), MTA+Zinc oxide nanoparticles and Biodentine+Zinc oxide Nano particles was examined using the broth dilution method (CLSI M07-A8). **(figure 1)**

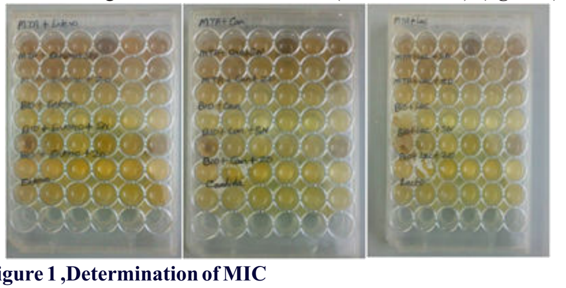


Figure 1 ,Determination of MIC

The MIC was determined in Brain heart infusion broth using broth dilutions methods with adjusted bacterial concentration (1×10^8 CFU/ ml, 0.5 McFarland's standard). The MIC is the lowest concentration of antimicrobial agents that visually inhibits the growth of microorganisms. The MIC was determined by the visual turbidity of the tubes before and after the incubation.

Membrane enclosed immersion test (MEIT)

Initially the materials were prepared freshly according to the manufacturer's instruction.Then the samples were subjected to MEIT assay in which prepared materials were enclosed within a sterile microbiologic paper. The material were wrapped in the membrane and then closed off at the top by using a sterile surgical thread, and each samples were immersed into the test wells of a 24-well plate containing 1 ml of bacterial suspension. Adhesive tape was used to secure the wrapped membranes to the plate containing the test cultures, **(figure 2)**.

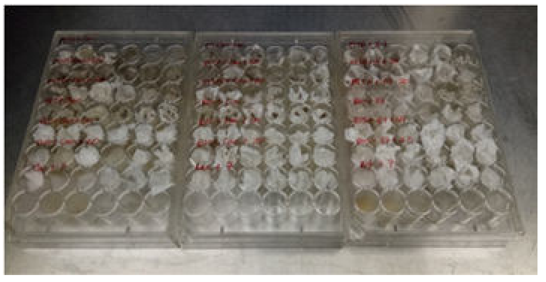


Figure 2 Membrane enclosed immersion test

In addition, 1 ml of the bacterial suspension in wells not containing MTA,Biodentine or Nanosilver and Zinc were served as controls. The plates were then incubated at 37°C under anaerobic conditions and then measured at 0.5, 1, 3, 48, and 72 h time points. The Optical density for each groups were measured using an ELISA reader at 570 nm All experiments were performed in triplicates, and the bacterial concentrations were reported as mean ± standard deviation (SD) mode. All data's were analyzed using T test and one way ANOVA by SPSS version 21. The *P* value lower than 0.05 was considered as statistically significant.

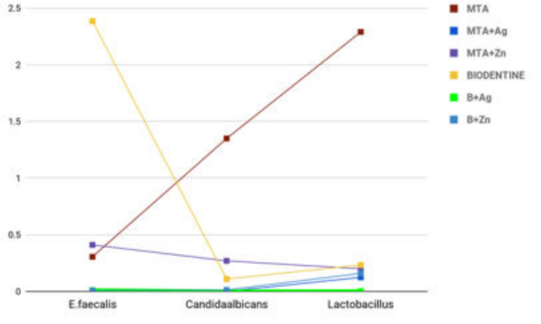
RESULTS:

Means and standard deviations values of different tested microorganisms are presented in **Table 1** .

Table 1, statistical analysis of data. P value lower than 0.05 is considered as statistically significant.

Micro Organisms	MTA	MTA+ Ag	MTA+Zn	BIODENTINE	B+Ag	B+Zn
E.faecalis	1.479	0.152	0.392	0.59	0.170	0.46
Candida albicans	1.976	0.174	0.463	0.20	0.001	0.981

Anti microbial property of different material tested is represented in line diagram, **Graph 1**



Graph 1. MTA and Biodentin with silver nanoparticles exhibited better antimicrobial property.

DISCUSSION

The bacteria and fungi selected in this study were either true endodontic pathogens or the species associated with therapy-resistant cases. E. faecalis is the aerobic facultative robust microorganism which is more likely found in root canals with persistent infection[9,10]. Another most commonly encountered fungal pathogen inside the infected root canals with persistent or refractory periapical lesions is the Candida albicans[11].

It is commonly stated that the antimicrobial activity of MTA is mainly associated with the elevated pH values. The Initial pH of this material is 10.2 and after 3 hours it increases upto 12.5[12]. It is known from various studies that the pH levels of approximately 12 could inhibit the growth of various microorganisms, including E. faecalis[13]. And also it is said that antifungal effect of MTA might be due to its higher pH or the substances like bismuth ions that are released from MTA into the media[14].

It is explained that the antimicrobial effect of biodentin is due to the excellent marginal integrity of the material by the formation of hydroxyapatite crystals at the surface of the cement. These crystals increase the sealing ability of the material especially at the interface with the dentinal walls. It is additionally stated that the small size and nanostructure of the gel that are formed of this calcium silicate cement is one of the factor that influences the sealability of the material to better spread over the surface of the dentine[15].

In our study, MTA Angelus along with the silver and Zinc oxide nanoparticles exhibited antibacterial effect against both the microorganisms. This result was concurrent with the study conducted by Al-Hezaimi et al, in which MTA exhibited antimicrobial effect against both *E. faecalis* and *C. albicans* Biodentin exhibited statistically more antifungal effect than MTA[16]. This was similar to the study conducted by Bhavana *et al.* who evaluated the antimicrobial effect of pro root MTA and Biodentine with disk diffusion method and found that biodentin exhibited better antimicrobial effect that MTA[17].

In our study silver exhibited more antibacterial effect that zinc oxide nanoparticle when mixed with both MTA and Biodentine. The exact mechanism of action of silver which known to exhibit antibacterial property is not fully understood. It is stated that the three possible theories that support the antibacterial effect of silver is that the silver ions destroy the cell wall, the silver ions interrupts the replication of RNA sequence thereby preventing the cellular multiplication and they act by blocking the cellular respiration by effectively suffocating the bacteria[18]. Another possible mechanism for the antibacterial action of silver ions is that its interaction with the thiol groups in proteins which enhances the inactivation of the bacterial proteins[19]. Literature states that the distinctive mechanisms behind the antibacterial action of Zinc oxide nano particles is that the direct contact of the particles with the cell wall which results in the destruction of the bacterial cell wall integrity[20].

CONCLUSION

Within the limitations of our study, it is stated that retrograde filling materials MTA Angelus and Biodentine along with silver and zinc oxide nanoparticles exhibited better antibacterial and antifungal effect. However MTA and Biodentine exhibited better antibacterial effect with silver nano particles than Zinc oxide nano particles against *E. faecalis* and Candida albicans.

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